updated search
11/22/02

```
=> s cat food
L1          611 CAT FOOD

=> s vitamin
L2       207990 VITAMIN

=> s l1 and l2
L3          213 L1 AND L2

=> s kibble or canned or cat treat
L4        14539 KIBBLE OR CANNED OR CAT TREAT

=> s l3 an dl4
MISSING OPERATOR L3 AN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l3 and l4
L5           83 L3 AND L4

=> s l5 and py<1999
L6           51 L5 AND PY<1999

=> s cat treat
L7           22 CAT TREAT

=> s l6 and l7
L8            0 L6 AND L7

=> s l6 and kibble
L9            7 L6 AND KIBBLE

=> d l9 1-7 ab bib kwic

L9   ANSWER 1 OF 7  USPATFULL
AB     A food composition having improved palatability to cats comprising a
       nutritious food mass and an palatability enhancing amount of a choline
       compound. The choline compound is incorporated within, or applied to
the
       surface of, the cat food composition.
AN     97:109554  USPATFULL
TI     Pet food composition of improved palatability and a method of enhancing
       the palatability of a food composition
IN     Lin, Charles F., Topeka, KS, United States
       Lin, Jack K., Topeka, KS, United States
       Jewell, Dennis E., Auburn, KS, United States
       Toll, Philip W., Vally Falls, KS, United States
       Stout, Neil P., Topeka, KS, United States
       Prewitt, Larry R., Auburn, KS, United States
PA     Colgate Palmolive Company, New York, NY, United States (U.S.
       corporation)
PI     US 5690988              19971125                              <--
AI     US 1996-594607          19960202 (8)
DT     Utility
FS     Granted
EXNAM  Primary Examiner: Czaja, Donald E.; Assistant Examiner: Koh, Choon P.
LREP   Shapiro, Paul
CLMN   Number of Claims: 20
ECL    Exemplary Claim: 1
DRWN   No Drawings
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LN.CNT 413
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5690988 19971125 <--
AB . . . palatability enhancing amount of a choline compound. The choline compound is incorporated within, or applied to the surface of, the **cat food** composition.
SUMM . . . preferences and require a high degree of palatability. Dry pet foods are widely marketed for cats. Generally, commercially sold dry **cat food** products have a relatively low moisture content of less than about 12% by weight and provide excellent nutrition. The lower. . . is generally well accepted by the cat but has the drawback that the product is significantly lower in palatability than **canned** or high moisture products that contain meat and have a moisture content above 50% by weight. One solution to the problem of low palatability of dry **cat foods** is to add a palatability enhancer to the food so that the cat will more readily accept the dry food. . . of the effectiveness of these materials, a need has continually existed for additives or ingredients that can be added to **cat food** products to further enhance the palatability of the product without reducing its nutritive properties.
SUMM The present invention provides a **cat food** composition of improved palatability wherein a palatability enhancing amount of a choline compound is incorporated within, or applied to the surface of, the **cat food** composition.
SUMM The use of choline compounds to enhance the palatability of **cat food** compositions has been found to be applicable to a wide range of commercial **cat food** products, and particularly dry **cat foods**.
SUMM . . . be demonstrated, when included internally at such concentration levels, choline chloride has little or no effect on palatability enhancement in **cat food**.
SUMM Various methods of adding the choline compound to **cat food** compositions may be employed in accordance with the practice of the present invention including: applying the choline compound uniformly mixed with other ingredients of the **cat food** during manufacture so that the choline compound forms a part of the basal food or topically applying the choline compound. . .
SUMM . . . its meaning all of the various properties of the food sensed by the cat such as taste and smell. The **cat food** compositions and methods of enhancing the palatability thereof to which the present invention is intended to apply generally relate to **cat food** compositions of any moisture content but preferably a **cat food** prepared from a nutritionally balanced mixture of proteinaceous and farinaceous ingredients having a moisture content of less than about 75%. . . It is presently believed, however, that the palatability enhancer of the present invention is especially important for use with dry **cat foods**, that is, foods having a moisture content of less than about 12% because of their relative need for some palatability. . .
SUMM The term "**cat food** composition", as used herein, is generally intended to apply to commercially sold, nutritionally balanced **cat food** compositions. **Cat food** compositions meeting this definition are characterized by a minimum protein content since there is a certain minimum protein level required

when the **cat food** composition provides the sole food intake for the cat. Commercially sold dry **cat food** compositions typically have a minimum protein content that is dependent upon the age of the animal to which it is. . . 25% by weight and more typically at least about 30% by weight on a 90% dry matter basis in the **cat food** product.

SUMM The **cat food** compositions of the present invention to which the choline compound is added are not intended to be restricted by any. . . desired as well as their availability to the pet food manufacturer. Generally, aside from the nutrition balancing additives such as **vitamins** and minerals, or other additives such as preservatives, emulsifiers, included in products of this type, commercial pet food compositions for. . .

SUMM The choline compound may be applied in accordance with the practice of the present invention, after manufacture of the **cat food**, in a palatability enhancing amount, to the surface of the **cat food** composition generally in an amount of about 0.06% by weight or more and preferably about 0.12 to about 0.60% by. . . most preferably about 0.24 to about 0.30% by weight. This level of choline compound provides a significant palatability improvement over **cat food** compositions of identical formulation to which the choline compound has not been topically applied to the food product surface.

SUMM When admixed with the other ingredients of the **cat food** during manufacture and present internally therein palatability enhancement, an amount of choline compound of at least about 0.25% by weight. . .

SUMM . . . be used in combination with other known palatability enhancers. For example, phosphoric acid, coated onto the surface of a dry **cat food**, has been shown to be a palatability enhancer. U.S. Pat. No. 3,679,429 discloses a method for improving the palatability of dry **cat food** by coating the food with fat and one of the following flavor enhancing acids: hexamic, tartaric, fumaric and lactic acids, phosphoric and citric acids. U.S. Pat. No. 3,930,031 discloses improving the palatability of semi-dry and dry **cat food** by coating the food with a mixture of phosphoric acid and citric acid wherein the coating provides at least 0.5%. . .

SUMM . . . application of the palatability enhancing amount of the choline compound as a coating applied topically to the surface of the **cat food**, it is preferred to apply the choline compound as a dispersion with a fat material such as choice white grease. . .

SUMM In one means of effecting the application of the choline compound to the surface of a **cat food** composition, according to the present method of enhancing the palatability thereof, food particles such as those of the extruded type. . . food particles to provide the desired level of fat and a palatability enhancing amount of the choline compound on the **cat food** particles. Following coating of the food particles, the coated particles are collected and then transported, if desired, to a tumbling. . .

SUMM If it is desired to incorporate the choline compound within the **cat food** product admixed with the other food product ingredients, the choline compound is merely admixed with the other

ingredients of the. . .

SUMM . . . or intimately admixed with the other food ingredients results in significant palatability response from cats in comparison to the same **cat food** composition without the choline compound. A significant improvement in **cat food** formulation is, therefore, achieved by the application of the choline compound since the palatability of the composition to the cat. . .

DETD **Cat Food** Composition Formulation Containing Choline Chloride Admixed With Other Food Ingredients

DETD An extruded **cat food** composition having a protein content of about 31% by weight on a 90% dry matter basis was prepared by mixing. . . animal by-product meal, fish meal, brewers rice and yellow corn and minor amounts of yeast, cellulose, fiber, salt (sodium chloride), **vitamins** and minerals.

DETD . . . under conditions of elevated temperature and pressure to form a continuous strand of product that was segmented into pieces or **kibbles** by a rotating cutting knife upon exit of the strand from the extruder. The particles were then conveyed to a forced air drying system and the moisture level reduced to below about 10% by weight. The dried, extruded **kibbles** were placed in a small cement mixer for mixing with choice white grease which was heated to about 122.degree. F. This mixture was stirred for about 5 minutes to achieve a uniform coating. The so coated **cat food** was then placed in polyethylene-lined bags and stored at room temperature for about two days before being tested for palatability.

DETD . . . with the exception that 0.24% by weight choline chloride was admixed with the other ingredients used to prepare the extruded **cat food** product.

DETD The palatability of the two **cat food** products containing respectively 0.24 and 0.30% by weight choline chloride was measured using a palatability test which determined the extent. . . had been added and B is the sum of the weight consumed from pan B containing a commercially available dry **cat food** sold for mature cats containing about 0.24% by weight choline chloride as a nutritional supplement, which served as a control.

DETD . . . Food Intake

Composition	%*	Ratio
0.24	23	0.35
0.30	68	0.63**

*% of cats preferring the food containing choline chloride over the commercial **cat food** product which served as a control.

**p < 0.01 (Data is statistically significant to a confidence level of 99%).

DETD The results recorded in Table I indicate that the palatability of dry **cat food** is significantly increased as compared to a commercially available **cat food** when the concentration of choline chloride is raised from 0.24% to 0.30% by weight levels.

DETD **Cat Food** Composition Containing Choline Chloride Incorporated in White Grease Surface Coating

DETD A commercially available dry **cat food** containing 0.24% by weight choline chloride as a nutrition supplement, served as the control. The results are recorded in Table. . .

DETD The results recorded in Table II show that when choline chloride is applied to the surface of **cat food** at concentration levels of 0.06% by weight or more, a substantial enhancement in the palatability of the food to cats. . .

DETD For purposes of contrast, the procedure of Example II was repeated except the commercial **cat food**, which contained 0.2% sodium chloride, was coated with choice white grease containing varying amounts of sodium chloride salt.

DETD The commercial **cat food** without salt addition to the white grease coating was used as a control. The results are recorded in Table III. . .

DETD The results recorded in Table III indicate that saltiness induced by sodium chloride salt significantly decreased the palatability of the **cat food**. Thus, the palatability enhancement obtained from the use of choline chloride is not attributed to any saltiness of the compound.

DETD . . . acid powder (99.9% purity), and ascorbic acid powder (99.9% purity) were included in the choice white grease coating. A commercial **cat food** containing 0.24% by weight choline chloride as a nutrition supplement served as the control. The results are recorded in Table. . .

L9 ANSWER 2 OF 7 USPATFULL

AB A palatability enhancer composition for pet food comprises a pyrophosphate salt or an acid phosphate salt. The palatability enhancer may also include at least one of the following: an organic acid, a flavor, and phosphoric acid.

AN 93:12324 USPATFULL

TI Flavor composition for pet food

IN Gierhart, Dennis L., High Ridge, MO, United States
Hogan, William C., Bridgeton, MO, United States

PA Applied Food Biotechnology, Inc., Fenton, MO, United States (U.S. corporation)

PI US 5186964 19930216 <--

AI US 1990-577114 19900904 (7)

DT Utility

FS Granted

EXNAM Primary Examiner: Penland, R. B.

LREP Leydig, Voit & Mayer

CLMN Number of Claims: 3

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 336

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5186964 19930216 <--

SUMM The makers of animal food, particularly **cat food**, have a long-standing desire to provide a pet food having a high degree of nutritional value, palatability, resistance to bacterial. . . or additives. Each of these attributes, in various degrees, may be found in the three categories of pet food: (1) **canned** or high moisture content products (greater than 50% moisture), which are typically all meat products, and, for this reason, are. . . (3) semi-dry or intermediate moisture content products (about 15% to 50% moisture), which generally have a nutritional value higher than **canned** food and are easier to package and more convenient to use, but may also support the growth of contaminating microorganisms. Semi-dry products are generally less palatable than **canned** food, but generally more palatable than dry food.

SUMM Phosphoric acid, coated onto the surface of a dry **cat**

food, has been shown to be a palatability enhancer. U.S. Pat. No. 3,679,429 discloses a method for improving palatability of dry **cat food** by coating pellets of the food with fat and one of the following flavor enhancing acids: 0.05% to 0.3% hexamic,. . . to 1.0% phosphoric, or 0.5% to 1.0% citric. U.S. Pat. No. 3,930,031 discloses improving the palatability of semi-dry and dry **cat food** by coating the food with a synergistic mixture of phosphoric acid and citric acid wherein the coating provides at least. . . an acid is known to accelerate the oxidation of fats, which, as noted above, are typically applied topically to dry **cat foods**. This problem may be overcome as shown in U.S. Pat. No. 4,215,149, which discloses a method for maintaining the palatability.

.

SUMM . . . flavor components, thus dramatically decreasing the effectiveness of a liquid flavor when present at levels where the acid could effect **cat food** palatability.

SUMM . . . animal tissue or meals; grains, such as corn, milo, alfalfa, wheat, soy, and the like; carbohydrates; fat, e.g., tallow; minerals; **vitamins**; and preservatives. It is intended that the invention is not to be limited to any specific recitation of food ingredients,.

.

SUMM Method for Coating **Cat Food**

SUMM **Kibbles**, for example, uncoated extruded basal **cat food** obtained from a pet food manufacturer, are typically placed in a convenient container for mixing, such as a small cement. . . fat, such as lard, critical animal fat or beef tallow, is heated to about 160.degree. F. and sprayed onto the **cat food** in any convenient manner to obtain a coating of the **kibbles**. The coating need not be a continuous layer yet any reasonable sample preferably exhibits a uniformity of coating. The **cat food** should be mixed during and for a few minutes after spraying the fat to improve uniformity of the coating, although. . . A dry flavor is typically dusted on, preferably through a mesh screen to make the application more uniform on the **kibbles**, while the product is mixing. Alternatively, a flavor could be mixed with the fat and applied concurrently.

DETD Various liquid and dry **cat food** compositions according to the invention were compared with a variety of conventional **cat food** compositions in order to determine their relative palatability. Tests A-D compare compositions according to the invention containing sodium pyrophosphate to. . .

CLM What is claimed is:

1. A method of increasing the palatability of an extruded dry **cat food** composition comprising topically applying to said **cat food** a palatability enchancer composition consisting essentially of from about 0.1 to about 99%, by weight, of sodium acid pyrophosphate, in. . .

L9 ANSWER 3 OF 7 USPATFULL

AB A method is described for preventing diet-induced Carnitine deficiency in domesticated dogs and cats. A daily prophylactic amount of gamma-butyrobetaine is administered to the pet either as a dietary supplement in an amount of 1.0 to 5.0 grams of gamma-butyrobetaine per day, or gamma-butyrobetaine is provided as an additional ingredient to a commercial pet food in an amount of 1.0 to 5.0 grams of gamma-butyrobetaine per kilogram pet food.

AN 91:54596 USPATFULL

TI Method for preventing diet-induced carnitine deficiency in domesticated dogs and cats
IN Shug, Austin L., 1201 Shorewood Blvd., Madison, WI, United States 53705
Keene, Bruce W., North NC State University College of Veterinary Medicine, 4700 Hillsbourough St., Raleigh, NC, United States 27606
PI US 5030458 19910709 <--
AI US 1989-441110 19891127 (7)
DCD 20061128
DT Utility
FS Granted
EXNAM Primary Examiner: Penland, R. B.
LREP Gulbrandsen, Carl E.
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 280
PI US 5030458 19910709 <--
SUMM Pets, particularly the carnivores, are at great risk for developing L-Carnitine deficiencies. As Table 1 indicates, dog and **cat foods** are extremely low in free L-Carnitine levels as compared with that found in raw ground beef. Most pets are maintained. . .
SUMM . . . 53.6
GAINES GRAVY TRAIN BEEF FLAVOR 5 LBS
89.4
KALKAN MEALTIME SMALL CRUNCHY BITS 5 LBS
105.9
KEN-L-RATION LOVE ME TENDER CHUNKS-BEEF
27.3
KEN-L-RATION **KIBBLES** 'N BITS 4 LBS
78.6
PETTS BRAND ALL NATURAL (HUBBARD) 4 LBS
167.7
PURINA DOG CHOW 5 LBS 161.0
PURINA CHUCKWAGON DOG CHOW. . . 93.2
PURINA BUTCHER'S BLEND BEEF, BACON, LIVER
106.3
PURINA FIT AND TRIM 4.5 LBS 103.9
PURINA PUPPY CHOW 5 LBS 136.0
NUTRO MAX PUPPY **KIBBLE** PUPPY FOOD
143.5
NUTRO MAX **KIBBLE** DOG FOOD 192.7
IAMS MINI CHUNKS 182.9
EUKANUBA (BY IAMS) 216.3
** SAMPLE TYPE: SEMI-MOIST DOG FOOD
GAINES BURGERS - BEEF 36 OZ 55.5
KEN-L-RATION SPECIAL CUTS 24 OZ 59.2
** SAMPLE TYPE: **CANNED** DOG FOOD
ALPO BEEF & LIVER DINNER 14 OZ 222.8
ALPO LAMB CHUNKS 89.2
CARNATION MIGHTY DOG BEEF 6.5 OZ 1799.1
CARNATION MIGHTY DOG. . . BEEF, LIVER DINNER
33.9
KEN-L-RATION CHICKEN DINNER 30.2
RECIPE HEARTY MEAT DINNER 14 OZ 95.5
VETS-BEEF FLAVORED 15 OZ 62.5
** SAMPLE TYPE: DRY **CAT FOOD**
KALKAN CRAVE 18 OZ 135.7
CARNATION FRISKIES OCEAN FISH FLAVOR
168.6

STARKIST 9 LIVES CRUNCHY MEALS REAL TUNA & EGG
114.0
IAMS **CAT FOOD** 26 OZ 196.9
PURINA CAT CHOW 22 OZ 109.1
PURINA KITTEN CHOW 18 OZ 121.4
PURINA MEOW MIX 18 OZ 61.2
PURINA TENDER VITTLES MOIST CHICKEN DINNER
127.8
PURINA THRIVE 18 OZ 95.2
PURINA SPECIAL DINNERS SEA NIP DINNER 18 OZ
188.2
** SAMPLE TYPE: **CANNED CAT FOOD**
STARKIST AMORE TURKEY & GIBLET DINNER 3 OZ
94.0
STARKIST AMORE POACHED SALMON DINNER 3 OZ
101.2
CARNATION FRISKIES BUFFET TURKEY & GIBLET. . .
DETD . . . be combined with other valuable nutritional or prophylactic substances. Examples of this would be a combination of L-Carnitine with a **vitamin** and mineral preparation. Another example would be the inclusion of a prophylactic amount of L-Carnitine with an anti-heartworm medication such. . .
CLM What is claimed is:
. . . described in claim 1, wherein said gamma-butyrobetaine is administered by adding said prophylactic amount of gamma-butyrobetaine to a dog or **cat food** so as to form a mixture and daily feeding said mixture to said dog or cat.

L9 ANSWER 4 OF 7 USPATFULL
AB A method is described for increasing the plasma L-Carnitine level in pets. A daily prophylactic amount of L-Carnitine is administered in the pet either as a dietary supplement in an amount of 0.2 to 2.0 grams of L-Carnitine per day, or L-Carnitine is provided as an additional ingredient to a commerical pet food in an amount of 0.2 to 2.0 grams of L-Carnitine per kilogram pet food.
AN 89:95617 USPATFULL
TI Method for preventing diet induced carnitine deficiency in domesticated dogs and cats
IN Shug, Austin L., 1201 Shorewood Blvd., Madison, WI, United States 53705
Keene, Bruce W., 625 N. Blackhawk Ave., Madison, WI, United States 53705
PI US 4883672 19891128 <--
AI US 1988-187870 19880429 (7)
DT Utility
FS Granted
EXNAM Primary Examiner: Penland, R. B.
LREP Gulbrandsen, Carl E.
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 225
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 4883672 19891128 <--
SUMM Pets, particularly the carnivores, are at great risk for developing L-Carnitine deficiencies. As Table 1 indicates, dog and **cat foods** are extremely low in free L-Carnitine levels as compared with that found in raw ground beef. Most pets are maintained. . .
SUMM . . . 53.6

GAINES GRAVY TRAIN BEEF FLAVOR 5 LBS
89.4
KALKAN MEALTIME SMALL CRUNCHY BITS 5 LBS
105.9
KEN-L-RATION LOVE ME TENDER CHUNKS-BEEF
27.3
KEN-L-RATION **KIBBLES** 'N BITS 4 LBS
78.6
PETTS BRAND ALL NATURAL (HUBBARD) 4 LBS
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PURINA DOG CHOW 5 LBS 161.0
PURINA CHUCKWAGON DOG CHOW. . . 93.2
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106.3
PURINA FIT AND TRIM 4.5 LBS 103.9
PURINA PUPPY CHOW 5 LBS 136.0
NUTRO MAX PUPPY **KIBBLE** PUPPY FOOD 143.5
NUTRO MAX **KIBBLE** DOG FOOD 192.7
IAMS MINI CHUNKS 182.9
EUKANUBA (BY IAMS) 216.3
** SAMPLE TYPE: SEMI-MOIST DOG FOOD
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KEN-L-RATION SPECIAL CUTS 24 OZ 59.2
** SAMPLE TYPE: **CANNED** DOG FOOD
ALPO BEEF & LIVER DINNER 14 OZ 222.8
ALPO LAMB CHUNKS 89.2
CARNATION MIGHTY DOG BEEF 6.5 OZ 1799.1
CARNATION MIGHTY DOG. . . BEEF, LIVER DINNER
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114.0
IAMS **CAT FOOD** 26 OZ 196.9
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PURINA KITTEN CHOW 18 OZ 121.4
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PURINA SPECIAL DINNERS SEA NIP DINNER 18 OZ
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94.0
STARKIST AMORE POACHED SALMON DINNER 3 OZ
101.2
CARNATION FRISKIES BUFFET TURKEY & GIBLET. . .
DETD . . . be combined with other valuable nutritional or prophylactic substances. Examples of this would be a combination of L-Carnitine with a **vitamin** and mineral preparation. Another example would be the inclusion of a prophylactic amount of L-Carnitine with an anti-heartworm medication such. . .

L9 ANSWER 5 OF 7 USPATFULL

AB A dried cereal based pet food composed of farinaceous and proteinaceous materials encapsulated within a glazed coating composed of liver and farinaceous material, the combination of which has a synergistic effect that excites the taste buds of the pet more than either of the highly palatable ingredients taken separately. In a preferred form, the matrix of farinaceous and proteinaceous material is formed to provide a core of desired shape and baked to less than 18% moisture by weight. While the shaped matrix is extremely hot, i.e., having an internal temperature in excess of 200.degree. F., it is immersed in a meaty coating of farinaceous material and liver having a lower temperature. A portion of the coating material is thereafter trapped within the core by a second baking that forms a hard crust of glazed, dried meaty substance on the exterior to encapsulate the matrix. In a further preferred embodiment the core is formed of uncooked farinaceous material combined with finely divided flakes of uncooked meat or meat by-product.

AN 82:62889 USPATFULL

TI Glazed liver coated biscuit or **kibble** for pets

IN Brown, Bruce W., P.O. Box 600, Medina, TX, United States 78055
Copple, Virgil E., 233 Gruene Rd., New Braunfels, TX, United States 78130
Wilson, Carroll K., 1128 Winston, El Paso, TX, United States 79907

PI US 4366175 19821228 <--

AI US 1980-172216 19800725 (6)

RLI Continuation-in-part of Ser. No. US 1978-927798, filed on 25 Jul 1978, now patented, Pat. No. US 4229485

DT Utility

FS Granted

EXNAM Primary Examiner: Bernstein, Hiram

LREP Fulwider, Patton, Rieber, Lee & Utecht

CLMN Number of Claims: 5

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 569

TI Glazed liver coated biscuit or **kibble** for pets

PI US 4366175 19821228 <--

SUMM . . . the invention pertains includes the field of edible material, particularly pet food in the form of bone-shaped biscuits, cookies or **kibbles**.

SUMM . . . in nonrefrigerated containers without the need of hermetically sealed containers. The product can be provided in the form of small **kibbles** or, as preferred, in greater than bite-size, for example in the form of large, molded bone-shaped pieces. Particularly in the. . .

SUMM Dog and **cat foods** are commonly prepared as either meal-type rations or **canned**-type rations and are commonly formulated from a combination of proteinaceous and farinaceous materials. The proteinaceous material is derived from either. . .

SUMM **Canned**-type animal foods having a meat-like texture and high-moisture content are generally received quite favorably by animals, apparently due in part. . .

SUMM . . . heretofore been provided an animal food product which has both the uniform high palatability associated with the meaty texture of **canned** food and the convenience and high nutritional value associated with meal-type foods. The present invention provides such products.

SUMM Whether being applied to **kibble**-like pieces or to the

preferred baked combination of uncooked farinaceous material and finely cut flakes of uncooked meat or meat. . .

SUMM . . . hereinafter. The blend of farinaceous material and meat or meat by-product comprising the core may be in the form of **kibble**-like pieces or otherwise. When in **kibble** form, it may be prepared by extrusion through conventional cold forming extrusion equipment to form pieces, for example one inch. . .

SUMM . . . and to aid in preserving the product. Up to 20% animal fat can be added if desired. Additives such as **vitamins**, minerals and coloring matter may be added at the blending stage although as a result of using the meat or. . .

SUMM The core material, whether **kibble**-like in size and form or whether molded as biscuits, is coated with a slurry of comminuted liver.

In this regard,. . .

DETD

Ingredients	Percent by Weight
Whole ground wheat	54.84
Meat and bone meal	9.25
Whole ground corn	2.96
Bone regrind	2.77
Salt	1.48
Potassium sorbate	0.02
Vitamin and mineral premix	0.10
Water	28.67

L9 ANSWER 6 OF 7 USPATFULL

AB Methods for forming simulated, shaped, edible products suitable for human and/or animal consumption, or as a bait for marine creatures; and simulated, edible products produced thereby. More particularly, improved processes of the foregoing character wherein an aqueous alginate solution containing one of a sterilant or a sterilant neutralizer, with or without other additives such, for example, as comminuted food products, offal, coloring materials, flavorings, attractants and/or species specific repellants or irritants for marine creatures, are mixed into an essentially homogeneous viscous solution with the viscous solution thus formed being introduced in a desired shaped configuration into a setting bath containing a metallic salt in aqueous solution wherein the setting bath includes the other of the sterilant or sterilant neutralizer, so as to: (i) substantially instantaneously "set" the shaped viscous solution upon contact with the setting bath in the shaped configuration in which it is introduced; (ii) sterilize the ingredients comprising the product thus formed; and (iii), neutralize the sterilant so as to permit the intended use of the product without danger of harm or irritation from hazardous residues; yet, wherein the product thus formed can be readily made indistinguishable from the real or natural product that it simulates in terms of appearance, texture, bite, taste, feel, olfactory and/or gustatory characteristics, and other sensory characteristics.

AN 82:59213 USPATFULL

TI Method for forming shaped products for human and/or animal consumption or as marine bait and products produced thereby
IN Cox, James P., Lynden, WA, United States
PA Wells, Loyal, Thiensville, WI, United States (U.S. individual)
Cox Family Laboratories, Inc., United States (U.S. corporation) a part interest
PI US 4362748 19821207 <--
AI US 1980-193434 19801003 (6)
DT Utility
FS Granted
EXNAM Primary Examiner: Yoncoskie, Robert A.
LREP Hughes, Barnard & Cassidy
CLMN Number of Claims: 38
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 1039
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 4362748 19821207 <--
DETD . . . example, as meat, fish and/or cereal products, meat or fish by-products, meat or fish offal, other proteinaceous materials and/or nutrients, **vitamin** supplements, etc., with the particular selection of additives being optional and dependent upon the nature of the product being produced.. . . FIG. 13 or 14, or a screw-type auger
delivery system would, for example, be satisfactory for forming edible pet food **kibbles** or the like; whereas the system of FIG. 8 would be satisfactory for forming edible caviar or the like.
DETD . . . discharging a continuous batter strand directly into the setting bath; and, the "set" product was thereafter cut up to form **kibbles**.
DETD EXAMPLE II--**CAT FOOD**
DETD Again employing the same process and equipment described in connection with Example I, a simulated **cat food** was prepared in accordance with the invention using the following ingredients in the indicated proportions:
CLM What is claimed is:
30. The method set forth in claim 28 wherein the product produced is simulated kibbled **cat food**.

L9 ANSWER 7 OF 7 USPATFULL
AB A dried cereal based pet food composed of farinaceous and proteinaceous materials encapsulated within a glazed coating composed of liver and farinaceous material, the combination of which has a synergistic effect that excites the taste buds of the pet more than either of the highly palatable ingredients taken separately. In a preferred form, the matrix of farinaceous and proteinaceous material is formed to provide a core of
desired shape and baked to less than 18% moisture by weight. While the shaped matrix is extremely hot, i.e. having an internal temperature in excess of 200.degree. F., it is immersed in a meaty coating of farinaceous material and liver having a lower temperature, causing a sudden drop in temperature which creates a partial vacuum in the inner core of the shaped matrix, drawing the aroma and flavor of the coating material into the matrix, which is then trapped within the core by a second baking that forms a hard crust of glazed, dried meaty substance on the exterior to encapsulate the matrix. In a further preferred embodiment the core is formed of uncooked farinaceous material combined with finely divided flakes of uncooked meat or meat by-product.
AN 80:52605 USPATFULL

TI Glazed liver coated biscuit or **kibble** for pets
IN Brown, Bruce W., El Paso, TX, United States
Copple, Virgil E., El Paso, TX, United States
Wilson, Carroll K., El Paso, TX, United States
PA Jerky Treats, Inc., El Paso, TX, United States (U.S. corporation)
PI US 4229485 19801021 <--
AI US 1978-927798 19780725 (5)
DT Utility
FS Granted
EXNAM Primary Examiner: Penland, R. B.
LREP Nilsson, Robbins, Dalgarn, Berliner, Carson & Wurst
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 559
TI Glazed liver coated biscuit or **kibble** for pets
PI US 4229485 19801021 <--
SUMM . . . the invention pertains includes the field of edible material, particularly pet food in the form of bone-shaped biscuits, cookies or **kibbles**.
SUMM . . . in nonrefrigerated containers without the need of hermetically sealed containers. The product can be provided in the form of small **kibbles** or, as preferred, in greater than bite-size, for example in the form of large, molded bone-shaped pieces. Particularly in the.
.
.
SUMM Dog and **cat foods** are commonly prepared as either meal-type rations or **canned**-type rations and are commonly formulated from a combination of proteinaceous and farinaceous materials. The proteinaceous material is derived from either. . .
SUMM **Canned**-type animal foods having a meat-like texture and high-moisture content are generally received quite favorably by animals,
apparently due in part. . .
SUMM . . . there has not been provided an animal food product which has the high palatability associated with the meaty texture of **canned** food and the convenience and high nutritional value associated with meal-type foods. The present invention provides such products. In particular,. . .
SUMM Whether being applied to **kibble**-like pieces or to the preferred baked combination of uncooked farinaceous material and finely cut flakes of uncooked meat or meat. . .
SUMM . . . the general form, the cores can be made of any known material and when it is in the form of **kibble**-like pieces, it can be made entirely of farinaceous materials. See for example the core materials of U.S. Pat. No. 3,808,340, incorporated herein by reference. Preferably, even when in **kibble** form, the core material is obtained by blending farinaceous material and meat or meat by-product described hereafter. **Kibble** can be prepared by extrusion through conventional cold forming extrusion equipment to form pieces, for example one inch long and. . .
SUMM . . . and to aid in preserving the product. Up to 20% animal fat can be added if desired. Additives such as **vitamins**, minerals and coloring matter may be added at the blending stage although as a result of using the meat or. . .
SUMM The core material, whether **kibble**-like in size and form or whether molded as biscuits, is coated with a slurry of comminuted liver.
In this regard,. . .

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NEWS 5	Apr 23	Search Derwent WPINDEX by chemical structure
NEWS 6	Apr 23	PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
NEWS 7	May 07	DGENE Reload
NEWS 8	Jun 20	Published patent applications (A1) are now in USPATFULL
NEWS 9	JUL 13	New SDI alert frequency now available in Derwent's DWPI and DPCI
NEWS 10	Aug 23	In-process records and more frequent updates now in MEDLINE
NEWS 11	Aug 23	PAGE IMAGES FOR 1947-1966 RECORDS IN CAPLUS AND CA
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DICTIONARY FILE UPDATES: 22 AUG 2001 HIGHEST RN 352422-14-1

TSCA INFORMATION NOW CURRENT THROUGH January 11, 2001

Please note that search-term pricing does apply when conducting SmartSELECT searches.

Structure search limits have been increased. See HELP SLIMIT for details.

```
=> s linolenic acid

          121 LINOLENIC
      5076815 ACID
         7651 ACIDS
      5082393 ACID
                (ACID OR ACIDS)
L1        109 LINOLENIC ACID
                (LINOLENIC(W)ACID)

=> s alpha linolenic acid/cn

L2          0 ALPHA LINOLENIC ACID/CN

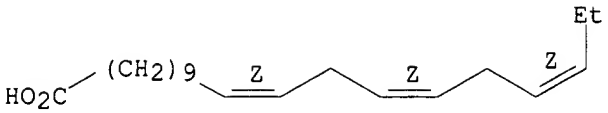
=> s alpha linolenic acid

      2266875 ALPHA
            6 ALPHAS
      2266875 ALPHA
                (ALPHA OR ALPHAS)
          121 LINOLENIC
      5076815 ACID
         7651 ACIDS
      5082393 ACID
                (ACID OR ACIDS)
L3          5 ALPHA LINOLENIC ACID
                (ALPHA(W)LINOLENIC(W)ACID)

=> d l3


L3   ANSWER 1 OF 5  REGISTRY  COPYRIGHT 2001 ACS
RN   17046-59-2  REGISTRY
CN   11,14,17-Eicosatrienoic acid, (11Z,14Z,17Z)- (9CI)  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   11,14,17-Eicosatrienoic acid, (Z,Z,Z)- (8CI)
OTHER NAMES:
CN   (all-cis)-.DELTA.11,14,17-Eicosatrienoic acid
CN   (all-cis)-11,14,17-Eicosatrienoic acid
CN   (Z,Z,Z)-11,14,17-Eicosatrienoic acid
CN   11,14,17-Eicosatrienoic acid
CN   11-cis,14-cis,17-cis-Eicosatrienoic acid
CN   11Z,14Z,17Z-Eicosatrienoic acid
CN   Bishomo-.alpha.-linolenic acid
CN   Homo-.alpha.-linolenic acid
FS   STEREOSEARCH
MF   C20 H34 O2
CI   COM
LC   STN Files:   AGRICOLA, BEILSTEIN*, BIOSIS, BIOTECHNO, CA, CAPLUS,
       CHEMCATS, EMBASE, TOXLINE, TOXLIT, USPATFULL
         (*File contains numerically searchable property data)

Double bond geometry as shown.
```



552 REFERENCES IN FILE CA (1967 TO DATE)
2 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
553 REFERENCES IN FILE CAPLUS (1967 TO DATE)

=> s eicosapentaenoic acid

806 EICOSAPENTAENOIC
5076815 ACID
7651 ACIDS
5082393 ACID
(ACID OR ACIDS)
L4 790 EICOSAPENTAENOIC ACID
(EICOSAPENTAENOIC(W)ACID)

=> s eicosapentaenoic acid/cn

L5 3 EICOSAPENTAENOIC ACID/CN

=> d

L5 ANSWER 1 OF 3 REGISTRY COPYRIGHT 2001 ACS
RN 32839-30-8 REGISTRY
CN Eicosapentaenoic acid, (Z,Z,Z,Z,Z)- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Eicosapentaenoic acid, (all-Z)-
OTHER NAMES:
CN (all-Z)-Eicosapentaenoic acid
CN cis-Eicosapentaenoic acid
CN **Eicosapentaenoic acid**
MF C20 H30 O2
CI IDS, COM
LC STN Files: ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CASREACT, CEN, CIN, EMBASE, PROMT, TOXLINE, TOXLIT, USPATFULL

CM 1

CRN 506-30-9
CMF C20 H40 O2

$HO_2C-(CH_2)_{18}-Me$

1002 REFERENCES IN FILE CA (1967 TO DATE)
28 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1004 REFERENCES IN FILE CAPLUS (1967 TO DATE)

=> s decosahexaenoic acid

0 DECOSAHEXAENOIC
5076815 ACID
7651 ACIDS
5082393 ACID
(ACID OR ACIDS)
L6 0 DECOSAHEXAENOIC ACID
(DECOSAHEXAENOIC(W)ACID)

```
=> s docosahexaenoic acid

          663 DOCOSAHEXAENOIC
      5076815 ACID
         7651 ACIDS
      5082393 ACID
                (ACID OR ACIDS)
L7        658 DOCOSAHEXAENOIC ACID
                (DOCOSAHEXAENOIC(W)ACID)

=> s docosahexaenoic acid/cn

L8          3 DOCOSAHEXAENOIC ACID/CN

=> d


L8   ANSWER 1 OF 3  REGISTRY  COPYRIGHT 2001 ACS
RN   32839-18-2  REGISTRY
CN   Docosahexaenoic acid, (Z,Z,Z,Z,Z,Z)- (9CI)  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   Docosahexaenoic acid, (all-Z)- (8CI)
OTHER NAMES:
CN   cis-Docosahexaenoic acid
CN   Docosahexaenoic acid
DR   179092-16-1
MF   C22 H32 O2
CI   IDS, COM
LC   STN Files:   ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA,
       CAPLUS, CASREACT, CEN, CIN, EMBASE, PROMT, TOXLINE, TOXLIT, USPATFULL

     CM   1

     CRN  112-85-6
     CMF  C22 H44 O2
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$HO_2C-(CH_2)_{20}-Me$

```
          1288 REFERENCES IN FILE CA (1967 TO DATE)
            31 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
          1289 REFERENCES IN FILE CAPLUS (1967 TO DATE)
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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.15       0.15

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FILE 'USPATFULL' ENTERED AT 13:09:17 ON 23 AUG 2001

=> s alpha linolenic acid and flaxseed oil

L1 91 ALPHA LINOLENIC ACID AND FLAXSEED OIL

=> s eicospataenoic acid and docosahexaenoic acid

3 FILES SEARCHED...
L2 0 EICOSPATAENOIC ACID AND DOCOSAHEXAENOIC ACID

=> s eicosapentaenoic acid and docosahexaenoic acid

L3 7151 EICOSAPENTAENOIC ACID AND DOCOSAHEXAENOIC ACID

=> s l1 and l3

L4 33 L1 AND L3

=> s omega-6 fatty acid or soy or safflower or canola

L5 69444 OMEGA-6 FATTY ACID OR SOY OR SAFFLOWER OR CANOLA

=> s l4 and l5

L6 11 L4 AND L5

=> s l6 and py<1999

2 FILES SEARCHED...
4 FILES SEARCHED...
L7 5 L6 AND PY<1999

=> dup rem l7

PROCESSING COMPLETED FOR L7
L8 2 DUP REM L7 (3 DUPLICATES REMOVED)

=> d l8 1-2 all

L8 ANSWER 1 OF 2 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 97132129 EMBASE
DN 1997132129
TI n-3 Fatty acids and serum lipoproteins: Human studies.
AU Harris W.S.
CS W.S. Harris, Metabolism/Vascular Res. Laboratory, Lipoprotein Research Laboratory, St Luke's Hospital, 4401 Wornall, Kansas City, MO 64111, United States. wharris@saint-lukes.org
SO American Journal of Clinical Nutrition, (1997) 65/5 SUPPL. (1645S-1654S).
Refs: 83
ISSN: 0002-9165 CODEN: AJCNAC
CY United States
DT Journal; Conference Article
FS 017 Public Health, Social Medicine and Epidemiology
029 Clinical Biochemistry
LA English
SL English
AB The effects of n-3 fatty acids from fish oils (**eicosapentaenoic acid** and **docosahexaenoic acid**) and plant oils (.**alpha**.-**linolenic acid**) on human serum lipids and lipoproteins are reviewed. Studies were included in this review

if they were placebo-controlled, crossover, or parallel design studies providing < 7 g n-3 fatty acids/d and with treatment periods of .ltoreq. 2
wk duration. Only three studies were available for evaluation of the effects of .**alpha**.-**linolenic acid** on serum
lipid concentrations. From these studies it appeared that .**alpha** .-**linolenic acid** (18:3n-3) was equivalent to n-6-rich
oils vis-a-vis lipid and lipoprotein effects. Only when very large amounts
of **flaxseed oil** were fed did the hallmark effect of
marine n-3 fatty acids- reduced triacylglycerol concentrations-appear. Thus, in terms of effects on lipoprotein metabolism, the plant-derived n-3
fatty acid is not equivalent to the marine-based acids. More studies using
the marine-based acids were examined and summarized. Both crossover (n = 36) and parallel (n = 29) design studies reached the same conclusions: total cholesterol is not materially affected by n-3 fatty acid consumption, low-density-lipoprotein cholesterol concentrations tend to rise by 5-10% and high-density- lipoprotein cholesterol by 1-3%, and serum
triacylglycerol concentrations decrease by 2530%. These effects of marine n-3 fatty acids are now well- established; what remains is to determine the mechanisms behind these effects and, more importantly, their health consequences.

CT Medical Descriptors:
*fat intake
clinical trial
conference paper
crossover procedure
human
lipid blood level
lipoprotein blood level
lipoprotein metabolism
Drug Descriptors:
*fatty acid
*fish oil
*lipid: EC, endogenous compound
*lipoprotein: EC, endogenous compound
*vegetable oil
cholesterol: EC, endogenous compound
docosahexaenoic acid
high density lipoprotein cholesterol: EC, endogenous compound
icosapentaenoic acid
linolenic acid
linseed oil
low density lipoprotein cholesterol: EC, endogenous compound
placebo
safflower oil
sunflower oil
triacylglycerol: EC, endogenous compound

RN (fish oil) 8016-13-5; (lipid) 66455-18-3; (cholesterol) 57-88-5; (**docosahexaenoic acid**) 25167-62-8, 32839-18-2;
(icosapentaenoic acid) 25378-27-2, 32839-30-8; (linolenic acid) 1955-33-5,
463-40-1; (linseed oil) 8001-26-1; (**safflower** oil) 8001-23-8;
(sunflower oil) 8001-21-6

L8 ANSWER 2 OF 2 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1
AN 95105782 EMBASE
DN 1995105782
TI **Alpha-linolenic acid** in the treatment of
rheumatoid arthritis: A double blind, placebo-controlled and randomized study: Flaxseed vs **safflower** seed.
AU Nordstrom D.C.E.; Honkanen V.E.A.; Nasu Y.; Antila E.; Friman C.; Konttinen Y.T.

CS Helsinki Univ. Central Hospital, Division of Rheumatology, Unioninkatu 38,FIN-00170, Helsinki, Finland
SO Rheumatology International, (1995) 14/6 (231-234).
ISSN: 0172-8172 CODEN: RHINDE
CY Germany
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
031 Arthritis and Rheumatism
037 Drug Literature Index
038 Adverse Reactions Titles
LA English
SL English
AB In rheumatoid arthritis various pro-inflammatory metabolites of arachidonic acid (AA), such as leukotriene B4 (LTB4) and prostaglandin E2 (PGE2), contribute to tissue destruction and pain. In contrast to AA, which is an **omega-6 fatty acid**, the omega-3 fatty acids, after having been liberated from the cell membrane phospholipids, are further converted into the non- or anti-inflammatory eicosanoids LTB5 and PGI3. AA concentration is an important regulatory step in the synthesis of both prostanoids and leukotriens. Dietary supplementation with **eicosapentaenoic acid** (EPA) and **docosahexaenoic acid** (DHA) has therefore been used to decrease the ratio of AA to EPA or DHA to obtain beneficial clinical effects. EPA and DHA are found in animal fat and are quite expensive compared to their precursor **alpha-linolenic acid** (alpha-LNA) found in **flaxseed oil**. We, therefore, performed a placebo-controlled trial with alpha-LNA in 22 patients with rheumatoid arthritis, using a linoleic acid preparation as a placebo. After a 3-month follow-up, the treatment group showed an increased bleeding time, but the clinical, subjective (global assessment, classification of functional status, joint score index, visual analogue scale, pain tendereness score) and laboratory parameters (haemoglobin, erythrocyte sedimentation rate, C-reactive protein) did not show any statistical alterations. AA, EPA and DHA did not change either in spite of a significant increase in alpha-LNA in the treatment group. Thus, 3-month's supplementation with alpha-LNA did not prove to be beneficial in rheumatoid arthritis.
CT Medical Descriptors:
*rheumatoid arthritis: DT, drug therapy
adult
arachidonic acid metabolism
article
bleeding time
clinical article
clinical trial
diarrhea: SI, side effect
diet supplementation
double blind procedure
erythrocyte sedimentation rate
female
human
intramuscular drug administration
joint function
male
oral drug administration
pain assessment
priority journal
randomized controlled trial
Drug Descriptors:
*linolenic acid: CT, clinical trial
*linolenic acid: AD, drug administration
*linolenic acid: DT, drug therapy

*linolenic acid: AE, adverse drug reaction
*linseed oil: AE, adverse drug reaction
*linseed oil: CT, clinical trial
*linseed oil: AD, drug administration
*linseed oil: DT, drug therapy
***safflower oil: AE, adverse drug reaction**
***safflower oil: DT, drug therapy**
***safflower oil: AD, drug administration**
***safflower oil: CT, clinical trial**
azathioprine: DT, drug therapy
corticosteroid: DT, drug therapy
corticosteroid: AD, drug administration
docosahexaenoic acid
fatty acid
gold: DT, drug therapy
gold: AD, drug administration
hydroxychloroquine: DT, drug therapy
icosapentaenoic acid
leukotriene b4: EC, endogenous compound
linoleic acid
membrane phospholipid: EC, endogenous compound
methotrexate: DT, drug therapy
nonsteroid antiinflammatory agent: DT, drug therapy
penicillamine: DT, drug therapy
placebo
prostaglandin e2: EC, endogenous compound
prostanoid: EC, endogenous compound
salazosulfapyridine: DT, drug therapy
RN (linolenic acid) 1955-33-5, 463-40-1; (linseed oil) 8001-26-1; (**safflower** oil) 8001-23-8; (azathioprine) 446-86-6; (**docosahexaenoic acid**) 25167-62-8, 32839-18-2; (gold) 7440-57-5; (hydroxychloroquine) 118-42-3, 525-31-5; (icosapentaenoic acid) 25378-27-2, 32839-30-8; (leukotriene b4) 71160-24-2; (linoleic acid) 1509-85-9, 2197-37-7, 60-33-3, 822-17-3; (methotrexate) 15475-56-6, 59-05-2, 7413-34-5; (penicillamine) 2219-30-9, 52-67-5; (prostaglandin e2) 363-24-6; (salazosulfapyridine) 599-79-1

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 NEWS  9  JUL 13   New SDI alert frequency now available in Derwent's
                   DWPI and DPCI

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               AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
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                                                         ENTRY    SESSION
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FILE LAST UPDATED: 21 Aug 2001 (20010821/ED)

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=> e omega 6 fatty acids

E1            4     OMEG/BI
E2       139086     OMEGA/BI
E3            0 --> OMEGA 6 FATTY ACIDS/BI
E4            1     OMEGA03/BI
E5           15     OMEGA1/BI
E6            1     OMEGA12/BI
E7            2     OMEGA2/BI
E8          572     OMEGA3/BI
E9            1     OMEGA440/BI
E10           1     OMEGA4403/BI
E11           1     OMEGA4499/BI
E12           2     OMEGA5/BI

=> e omega-6 fatty acids

E1            4     OMEG/BI
E2       139086     OMEGA/BI
E3            0 --> OMEGA-6 FATTY ACIDS/BI
E4            1     OMEGA03/BI
E5           15     OMEGA1/BI
E6            1     OMEGA12/BI
E7            2     OMEGA2/BI
E8          572     OMEGA3/BI
E9            1     OMEGA440/BI
E10           1     OMEGA4403/BI
E11           1     OMEGA4499/BI
E12           2     OMEGA5/BI

=> e omega-3

E1            4     OMEG/BI
E2       139086     OMEGA/BI
E3            0 --> OMEGA-3/BI
E4            1     OMEGA03/BI
E5           15     OMEGA1/BI
E6            1     OMEGA12/BI
E7            2     OMEGA2/BI
E8          572     OMEGA3/BI
E9            1     OMEGA440/BI
E10           1     OMEGA4403/BI
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E11          1     OMEGA4499/BI
E12          2     OMEGA5/BI

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=> s omega 3 fatty acid

L1        7858 OMEGA 3 FATTY ACID

=> s omega-3 fatty acid

L2        7858 OMEGA-3 FATTY ACID

=> s omega 6-fatty acid

L3        2687 OMEGA 6-FATTY ACID

=> s omega 6 fatty acid

L4        2687 OMEGA 6 FATTY ACID

=> s l1 and l4

L5        1498 L1 AND L4

=> s inflammation or inflammatory or antiinflammat?

L6      817222 INFLAMMATION OR INFLAMMATORY OR ANTIINFLAMMAT?

=> s l5 and l6

L7         203 L5 AND L6

=> s l7 and py<1999

   2 FILES SEARCHED...
   4 FILES SEARCHED...
L8         138 L7 AND PY<1999

=> s l8 and limolenic acid

L9           0 L8 AND LIMOLENIC ACID

=> s linolenic acid or flaxseed oil

L10      32779 LINOLENIC ACID OR FLAXSEED OIL

=> s l10 and l8

L11         42 L10 AND L8

=> s l11 and py<1999

   2 FILES SEARCHED...
   4 FILES SEARCHED...
L12         42 L11 AND PY<1999

=> dup rem l12

PROCESSING COMPLETED FOR L12
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L13 33 DUP REM L12 (9 DUPLICATES REMOVED)

=> d l13 1-33 all

L13 ANSWER 1 OF 33 USPATFULL
AN 1999:170640 USPATFULL
TI Hydrolysis-optimized lipid emulsions and use thereof
IN Pscherer, German, Melsungen, Germany, Federal Republic of
Junginger, Marco, Melsungen, Germany, Federal Republic of
Nehne, Jorg, Guxhagen, Germany, Federal Republic of
Carpentier, Yvon A., Brussel, Belgium
PA B. Braun Melsungen AG, Melsungen, Germany, Federal Republic of
(non-U.S.
corporation)
PI US 6008248 19991228
WO 9719683 19970605 <--
AI US 1998-43166 19980312 (9)
WO 1996-EP5184 19961123
19980312 PCT 371 date
19980312 PCT 102(e) date
PRAI DE 1995-19544310 19951128
DT Utility
FS Granted
REP US 5034414 Jul 1991 514/549.000 Wakabayashi et al.
US 5034415 Jul 1991 514/560.000 Rubin
US 5089268 Feb 1992 424/450.000 Katz
US 5444054 Aug 1995 514/054.000 Garleb et al.
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EP 298293 Jun 1988
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FR 2542613 Sep 1984
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REN Simoens et al. Manipulation of Tissue Fatty Acid Profile by Intravenous
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Quantitative Determination of Non-Esterified (or Free) Fatty Acids
(NEFA
or FFA) in Serum," Wako Chemicals GmbH, West Germany (1987).
EXNAM Primary Examiner: Harrison, Robert H.
LREP Christie, Parker & Hale, LLP
CLMN Number of Claims: 25
ECL Exemplary Claim: 1
DRWN No Drawings
AB The present invention pertains to hydrolysis-optimized isotonic lipid
emulsions comprising medium-chain triglycerides (MCT), vegetable oils
and fish oil, as well as their use for parenteral nutritition.

PARN This application is a Rule 371 of PCT/EP96/05184, filed Nov. 23, 1996.

SUMM The present invention pertains to hydrolysis-optimized isotonic lipid
emulsions (fat emulsions) for parenteral administration, in particular
for parenteral nutrition, and their use in situations of exaggerated

inflammatory response (e.g. post-surgery, post-trauma, sepsis, **inflammatory** or wasting diseases) or of increased risk of vascular thrombosis and severe cardiac arrytlmia where it is important to avoid inflicting an exogeneous triglyceride accumulation while making free fatty acids available to different tissues of the body as rapidly as possible.

Lipid emulsions for parenteral nutrition serve to supply the body with fats in an intravenously acceptable dosage form when normal (oral) nutrition is impossible, compromised or medically contraindicated or when it is necessary to promptly modify the fatty acid pattern of the cells. The lipid emulsions currently available are prepared from vegetable oils (e.g. safflower or soybean oils); in some cases they also contain medium-chain triglycerides (MCT) and/or oils of marine origin (fish oils).

Long-chain triglycerides of vegetable or marine origin serve as an energy source and, when containing polyunsaturated fatty acids, as suppliers of essential fatty acids. The classification of such polyunsaturated fatty acids into omega-6 or omega-3 series types is based on chemical structural features, more precisely, on the distance of the first unsaturated bond from the methyl end (omega end) of the fatty acid molecule.

The vegetable oils, e.g. of soybean and safflower, are characterized by a high content of polyunsaturated fatty acids of the omega-6 series (predominantly linoleic acid, 18:2 n-6) whereas their content of **omega-3 fatty acids** (almost exclusively in the form of .alpha.-**linolenic acid**, 18:3 n-3) is low.

Fish oils obtained from cold-water fish are characterized by a high content of polyunsaturated fatty acids of the omega-3 series (predominantly eicosapentaenoic acid, EPA, 20:5 n-3, and docosahexenoic acid, DHA, 22:6 n-3) whereas their content of **omega-6 fatty acids** is low.

The medium-chain triglycerides administered with the lipid emulsions serve mainly as a source of energy. Medium-chain triglycerides do not contain any unsaturated fatty acids and hence contain neither omega-6 nor omega-3 essential fatty acids.

Numerous clinical observations underline the principal suitability of lipid emulsions for parenteral nutrition and for substituting essential fatty acids in severe diseases and the metabolic conditions involved.

The human body is itself incapable of producing the vital, polyunsaturated long-chain fatty acids of the omega-6 or omega-3 series; i.e. they have to be administered orally, enterally or parenterally. The body is only able to synthesize longer-chain unsaturated fatty acids from shorter-chain ones; formation of **omega-6 fatty acids** from precursors of the omega-3 series or vice versa is impossible, however.

Correspondingly, there is a need for lipid emulsions for parenteral administration which contain medium-chain triglycerides as well as triglycerides of omega-6 and **omega-3 fatty acids** as lipid components.

EP-A-0 311 091 describes isotonic lipid emulsions for parenteral nutrition comprising, in addition to conventional additives and auxiliary agents, **omega-3 fatty**

acids, omega-3 fatty acids in the form of their esters or as components of fish oils, medium-chain triglycerides, as well as optionally at least one vegetable oil providing **omega-6 fatty acids** in a proportion of up to 30%, based on the lipid content of the emulsion.

DE-OS-37 21 137 describes lipid emulsions for parenteral nutrition comprising eicosapentaenoic acid triglyceride and/or docosahexaenoic acid triglyceride, or fish oils containing such triglycerides, as well as vegetable oils containing **omega-6 fatty acids**, and medium-chain triglycerides.

DE-OS-34 09 793 describes a lipid emulsion for transfusion comprising a fatty acid containing from 20 to 22 carbon atoms, esters thereof, or a mixture of 2 or more of such fatty acids or esters, as well as a vegetable oil, an emulsifier, and water. Said fatty acids are fatty acids from esters of marine origin (fish oils), in particular **omega-3 fatty acids**. Said vegetable oils are purified soybean and/or safflower oils.

In order that the exogeneous free fatty acids are made available to the body, they must either be released hydrolytically from the infused triglycerides by means of the enzyme lipoprotein lipase (LPL) or he taken up together with emulsion particles or their remnants directly into the cells. This initial step of lipidhydrolysis has long been considered the rate-determining step of lipid metabolism. This limitation arises from the relatively limited activity of lipoprotein lipase in cleaving triglycerides Thus, the maximum metabolizing rate for vegetable oil emulsions is about 3.8 g of lipid/kg body weight per day (Hallberg et al., Acta Physiol. Scand., Vol. 65, Suppl. 254 (1965), p. 2-23).

During triglyceride infusion, it is desirable, to achieve triglyceride serum concentrations which are as low as possible, e.g. corresponding to a low load of the reticulo-endothelial system (RES) by exogenous lipid.

Typically, post-operative and post-traumatic conditions as well as severe septic episodes are characterized by a substantial stimulation of the immune system. The immune response involves the release of cytokines (e.g. tumor necrosis factor and inter-leukins) which, at high levels, may cause severe tissue damage. In addition, high cytokine concentrations also impair hydrolysis of circulating triglycerides by LPL.

In such clinical conditions, it is of particular importance to use exogeneous triglycerides which are rapidly hydrolyzed and eliminated and which contain fatty acids (e.g. **omega-3 fatty acids**) capable of reducing cytokine production as well as cytokine toxicity on tissues.

Fatty acids as an energy substrate (for oxidative purposes) and for incorporation in membranes (for structural purposes) and as precursors of eicosanoids should also be made available to the body as quickly as possible.

Triglycerides typical of fish oils are hydrolyzed much more slowly than triglycerides from vegetable oils (e.g. soybean oil) which are themselves hydrolyzed more slowly than medium-chain triglycerides. Addition of a fish oil emulsion to a long-chain triglyceride emulsion can even inhibit hydrolysis of long-chain triglycerides (e.g. from soybean oil) by LPL.

Therefore, it is an object of the invention to provide a lipid emulsion for parenteral nutrition capable of being parenterally administered which has been optimized with respect to hydrolysis and elimination, which means that the triglycerides supplied with said lipid emulsion are hydrolyzed in the body extra- or intracellularly, i.e. cleaved to free fatty acids and glycerol, as quickly as possible without concomitant excessive increase of the serum level of free fatty acids. This implies that more lipids can be administered to the body parenterally within the same period of time without an increase of lipid concentration or concentration of hydrolysis products.

This object has been achieved by a hydrolysis-optimized isotonic aqueous lipid emulsion for parenteral administration comprising, based on the total lipid content of the lipid emulsion:

from 30% to 60% by weight of medium-chain triglycerides;

from 35% to 65% by weight of at least one vegetable oil comprising triglycerides which supply **omega-6 fatty acids**;

from 5% to 20% by weight of at least one fish oil comprising triglycerides which supply **omega-3 fatty acids**; and

conventional auxiliary agents and/or additives.

Surprisingly, it has been found that the object of the invention may be achieved by combining in the same emulsion particle medium-chain triglycerides, vegetable oils rich in **omega-6 fatty acids**, and fish oils containing **omega-3 fatty acids** in the quantitative proportion mentioned above. In particular, it has been found that the MCT/vegetable oil/fish oil mixtures of the invention are more quickly hydrolyzed than known MCT/vegetable oil mixtures and MCT/vegetable oil/fish oil mixtures of the prior art. Thus, triglyceride load of the body by exogeneous triglycerides is avoided. Medium-chain fatty acids and long-chain essential fatty acids become quickly available to the body. This involves no significant increase of the serum concentration of free fatty acids despite the fact that more lipids are supplied to the body per unit of time. Further, rapid incorporation of **omega-3 fatty acids** in platelet and leucocyte membrane phospholipids can be observed.

The lipid emulsions according to the invention include emulsified mixtures of oils (lipids) rather than mixtures of the emulsions.

According to the invention, those medium-chain triglycerides are used which have chain lengths of fatty acid ranging from C.sub.6 to C.sub.14 and which are comprised of at least 90% by weight of triglycerides of caprylic acid (C.sub.8) and capric acid (C.sub.10). The fraction of medium-chain triglycerides, based on the total lipid content of the lipid emulsion, is preferably from 45% to 55%, more preferably from 48% to 52%, by weight.

The lipid emulsions according to the invention further contain at least one vegetable oil containing triglycerides made predominantly of **omega-6 fatty acids**.

Preferred vegetable oils are safflower oil and/or soybean oil, the

content of such vegetable oils in the lipid emulsion preferably being from 35% to 45%, more preferably from 38% to 42%, by weight, based on the lipid content of the lipid emulsion. The vegetable oils contain triglycerides of fatty acids having chain lengths of C.sub.16 to C.sub.20 and predominantly contain triglycerides of **omega-6 fatty acids**.

Fish oils are known to contain eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) incorporated in triglycerides which, being so-called highly unsaturated **omega-3 fatty acids**, are essential building blocks which have to be supplied to the body and which are biologically important, for example, as precursors of eicosanoids and as structural elements of membrane lipids. These acids are further attributed antithrombotic and lipid-lowering actions. Since their isolation from natural products and their chemical synthesis is expensive, fish oils, being relatively inexpensive, are the suppliers of choice for such essential fatty acids.

As used in the invention, the term "fish oils" is intended to comprise natural fish oils, processed fish oils, or highly purified fish oil concentrates. According to the invention, processed fish oils may also be used, such as described e.g. in EP-A-0 298 293 which is incorporated herein by reference.

Suitable exemplary fish oils are oils which are obtained from cold water fish on a technically significant scale or oils which are synthetically obtainable by esterification of **omega-3-fatty acids** (obtained from fish oil of cold water fish, preferably salmon, sardine, mackerel, herring, anchovy, smelt and swordfish, by hydrolysis of the triglycerides and subsequent purification and concentration of the resultant **omega-3-fatty acids**) with glycerol. Fish oils generally contain triglycerides of fatty acids having chain lengths of from 12 to 22 carbon atoms. Particularly preferred are highly purified fish oil concentrates which are obtained, for instance, from sardine, salmon, herring and/or mackerel oils. They have an eicosapentaenoic acid content of from 20 to 40%, preferably at least 25%, based on the fatty acid methyl esters of the fish oil concentrate as determined by gas chromatography (percent by area). Furthermore, they have a docosahexaenoic acid content of from 10 to 20%, preferably at least 12%, based on the fatty acid methyl esters of the fish oil concentrate as determined by gas chromatography (percent by area). In case of the fish oils which are synthetically obtainable by the re-esterification of the **omega-3-fatty acids** the total concentration of eicosapentaenoic+docosahexaenoic acid can be at least 45% on basis of the triglycerides.

It is particularly preferred to use a fish oil rich in EPA when **inflammatory** processes are to be influenced. Fish oil rich in DHA is particularly preferred in pediatric patients in the case of **omega-3 fatty acid** deficiency to influence growth and maturation of the central nervous system.

Preferably, the content of fish oil, based on the total lipid content of the lipid emulsion, is from 10% to 20%, more preferably from 10% to 14%, by weight.

The total lipid content of the lipid emulsion is from 5% to 30%, preferably from 10% to 25%, by weight, based on the aqueous lipid emulsion.

In addition to distilled water, the isotonic lipid emulsion contains the usual auxiliary agents and/or additives, such as emulsifiers, emulsifying aids (co-emulsifiers), stabilizers. antioxidants, and isotonizing additives.

As emulsifiers, physiologically acceptable emulsifiers are used, such as phospholipids of animal or vegetable origin. Particularly preferred are purified lecithins, especially soybean lecithin, egg lecithin, or fractions thereof, or the corresponding phosphatides. The emulsifier content is from 0.6% to 1.5%, preferably 1.2%, by weight, based on the total emulsion.

Further, alkali metal salts of long-chain, C.sub.16 to C.sub.20, fatty acids may be used as emulsifying aids (co-emulsifiers). Especially preferred are their sodium salts. The co-emulsifiers are employed in concentrations of from 0.005% to 0.1%, preferably 0.02% to 0.04%, by weight, based on the total emulsion. Further, cholesterol or a cholesterol ester alone or in combination with other co-emulsifiers may be employed in a concentration of from 0.005% to 0.1%, preferably from 0.02% to 0.04%, by weight.

The lipid emulsion according to the invention may contain vitamin E, in particular .alpha.-tocopherol, and/or ascorbyl palmitate as antioxidants and thus for protection from peroxide formation in amounts of from 10 to 1000 mg, preferably 25 to 200 mg, based on 100 g of lipid.

For stabilization and isotonization, the emulsion according to the invention may contain from 2% to 5% by weight of a stabilizing or isotonizing additive, for example, a polyhydric alcohol. In this connection, glycerol, sorbitol, xylitol or glucose are preferred, glycerol being particularly preferred.

The lipid emulsions according to the invention are invariably oil-in-water (o/w) emulsions in which the outer, continuous phase consists of distilled water purified for parenteral purposes Such o/w emulsion is obtained by mixing MCT, vegetable oil and fish oil and subsequent emulsification. After sterilization, the lipid emulsion has a pH of from 6.0 to 9.0, preferably from 6.5 to 8.5.

The isotonic lipid emulsions according to the invention can be prepared by known procedures with inertization. The usual approach is first to mix the lipids, emulsifier and other auxiliary agents and additives and then to fill up with water with dispersing. The water may optionally contain additional water-soluble components (e.g. glycerol). The emulsion thus obtained still contains lipid particles having a diameter of about 10 .mu.m. The average droplet size of the emulsion must then further be reduced by additional homogenization, e.g. using a high-pressure homogenizer. For parenteral application, medium lipid droplet sizes of less than 1.0 .mu.m, in particular less than 0.5 .mu.m, are preferred.

The lipid emulsions according to the invention are used for parenteral administration, in particular parenteral nutrition, of patients with exaggerated **inflammatory** responses or increased risk of vascular thrombosis or severe cardiac arrythmia. In particular, the lipid emulsions according to the invention can be used with patients in post-operative and post-traumatic conditions or **inflammatory** diseases; further, e.g., in severe or persistent post-aggression metabolism following operations, such as abdominal operations or organ

transplantations, and multiple trauma, **inflammatory** diseases, burns, infections, impending or manifest sepsis, impaired respiratory function, conditions of excessive production of cytokines, wasting diseases, and increased risk of severe cardiac arrythmia (e.g. ventricular fibrillation) or vascular thrombosis. The lipid emulsion according to the invention can also be used for parenteral nutrition following shock conditions for improving microperfusion and metabolic performance of organs poorly supplied with blood in terms of metabolic reanimation.

The invention will be illustrated by the following examples.

DETD PREPARATIVE EXAMPLES

Table 1 shows the fatty acid composition (approx. %) of various oils used in the lipid emulsions of the following examples:

TABLE 1

Fatty acid	MCT oil.sup.1)	Soybean oil.sup.2)	Safflower oil.sup.3)	Fish oil.sup.4)
6:0	<2	--	--	--
8:0	64	--	--	--
10:0	34	--	--	--
12:0	<3	--	--	<1
14:0	<1	--	--	5
16:0	--	11	7	10
16:1	--	--	--	7
16:2	--	--	--	1
16:3	--	--	--	1
16:4	--	--	--	3
18:0	--	4	3	1
18:1	--	22	14	10
18:2 n-6	--	55	75	2
18:3 n-3	--	8	<1	1
18:4 n-3	--	--	--	4
20:0	--	<1	<1	--
20:1	--	<1	<1	2
20:4 n-6	--	--	--	2
20:5 n-3	--	--	--	28
22:1	--	--	--	1
22:4	--	--	--	<1
22:5	--	--	--	3
22:6 n-3	--	--	--	13
.SIGMA. n-6	--	55	75	4
.SIGMA. n-3	--	8	<1	46
n-6:n-3	--	7:1	.gtoreq.75:1	1:12

.sup.1) medium chain triglycerides, e.g. Captex 355, commercial product o Karlshamns.
.sup.2) soybean oil, e.g. Sojaol, commercial product of Croda.
.sup.3) safflower oil, e.g. Saflorol, commercial product of Gustav Heess.
.sup.4) highly purified fish oil, e.g. Sanomega S2BGA, commercial product of Nippon Oil and Fats.

Mixture I containing MCT, vegetable oil, fish oil, emulsifier (fractionated phospholipids from chicken egg yolk) is dispersed by means of Ultra-Turrax and filled up with aqueous component II with stirring. The pH value is adjusted to pH 8.0 to 9.0 using an aqueous sodium hydroxide solution and/or sodium oleate. Subsequent homogenization is performed in a high-pressure homogenizer at 400 kg/cm.sup.2. After dispensing in glass bottles of appropriate grade, heat sterilization is

performed by known methods.

TABLE 2

Preparative Example	1 (comparative example 1*)	2	3	4	5 (comparative example 2**)
I.					
medium-chain triglycerides	1000 g	500 g	1000 g	1000 g	1000 g
from partial synthesis					
purified safflower oil	--	--	800 g	--	
purified soybean oil	1000 g	400 g	--	800 g	600 g
highly purified fish oil	--	100 g	200 g	200 g	400 g
cholesterol acetate	--	--	2 g	--	
purified phospholipids from:	120 g egg	90 g egg	120 g egg	120 g egg	120 g egg
.alpha.-tocopherol	2000 mg	1000 mg	2000 mg	2000 mg	2000 mg
ascorbyl palmitate	1500 mg	--	1000 mg	1500 mg	1500 mg
sodium oleate	3,0 g	2,5 g	--	3,0 g	3,0 g
II. glycerol	250 g	250 g	250 g	250 g	250 g
NaOH to pH 8.0-9.0	--	--	to pH	--	--
water for injections ad	10 I	10 I	10 I	10 I	10 I

*MCT/vegetable oil (50:50)
**MCT/vegetable oil/fish oil (50:30:20) according to EPA-O 311 091

A sterile and pyrogen-free, stable emulsion resulted containing lipid droplets having an average size of less than 0.5 .mu.m with a shelf-life at room temperature of at least 18 months.

EXAMPLE 1

(in vivo)

1. Determination of Triglyceride Hydrolysis

Eight male subjects (age (av. .+-.st.d.) 23.+-.3 years) were infused with a lipid emulsion of MCT/vegetable oil (50:50) over 5 h each on 4 successive days (treatment A, table 3; preparative example 1 in table 2). After an interval of four weeks, a lipid emulsion of MCT/vegetable oil/fish oil (50:40:10) was infused under the same conditions (treatment B, table 4; preparative example 4 in table 2). After another interval of at least eight weeks, a lipid emulsion of MCT/vegetable oil/fish oil (50:30:20) was infused under the same conditions (treatment C, table 5; preparative example 5 in table 2). Triglyceride hydrolysis in the serum (measured as the average infusion rate in mg of lipids/kg body weight/h under triglyceride clamp conditions at a serum concentration of 3.0 mmol/l from 3rd to 5th hours of infusion, 9 measurements per subject and per day; analysis of variance) was determined as follows:

TABLE 3

Treatment A (Comparative Example 1)
Average infusion rate (3rd to 5th hour) with an MCT/vegetable oil (50:50) emulsion [mg of lipids/kg body weight/h]

Subject	Day 1	Day 2	Day 3

1.	171	155	180
2.	98	103	101
3.	142	161	122
4.	180	175	166
5.	182	223	243
6.	203	259	269
7.	129	129	143
8.	188	221	170
average .+-. st.d.	162 .+-. 35	178 .+-. 53	174 .+-. 57

TABLE 4

Treatment B (according to the invention)
Average infusion rate (3rd to 5th hour) with an MCT/vegetable oil/fish oil (50:40:10) emulsion [mg of lipids/kg bodyweight/h]

Subject	Day 1	Day 2	Day 3
1.	224	236	203
2.	201	134	163
3.	186	199	182
4.	190	201	179
5.	255	278	273
6.	259	272	271
7.	147	154	142
8.	176	182	181
average .+-. st.d.	205 .+-. 39	207 .+-. 52	199 .+-. 48

TABLE 5

Treatment C (Comparative Example 2)
Average infusion rate (3rd to 5th hour) with an MCT/vegetable oil/fish oil (50:30:20) emulsion [mg of lipids/kg body weight/h]

Subject	Day 1	Day 2	Day 3
1.	202	192	186
2.	133	122	120
3.	147	148	174
4.	228	211	204
5.	233	241	231
6.	168	250	259
7.	147	189	161
8.	174	177	188
average .+-. st.d.	179 .+-. 36	191 .+-. 41	190 .+-. 40

Triglyceride hydrolysis under treatment B according to the invention was significantly higher than that under treatments A ($p<0.0001$) and C ($p<0.05$) for all days of treatment. Thus, the average infusion rate over three days was 4.9 g of triglycerides/kg body weight/day for the lipid emulsion of MCT/vegetable oil/fish oil (50:40:10), and 4.1 and 4.5 g of triglycerides/kg body weight/day, respectively, for the lipid emulsions of MCT/vegetable oil (50:50) and MCT/vegetable oil/fish oil (50:30:20). The lipid emulsions composed according to preparative examples 2 and 3 give similar results. The result of a more rapid hydrolyzation of the lipid emulsions according to the invention to give free fatty acids as compared to the concentional lipid emulsions of the prior art can also be confirmed by in vitro studies (cf. example 2).

2. Determination of the Level of Free Fatty Acids in the Serum

The level of free fatty acids in the serum of the subjects was determined on the days of treatment before (0 h) and immediately

following (5 h) administration of the lipid emulsion. A suitable test for this purpose is, for instance, NEFAC test (an in vitro enzymatic colorimetric method) of Wako Chemicals GmbH, Germany.

It has been found that upon administration of the lipid emulsion of MCT/vegetable oil/fish oil (50:40:10) according to the invention the serum concentrations of free fatty acids are not increased to markedly higher values as compared to administration of a commercial lipid emulsion of MCT/vegetable oil (50:50) and another lipid emulsion of MCT/vegetable oil/fish oil (50:30:20) although more lipids have been supplied to the body per unit of time. The experimental results are given hereinafter in tables 6 and 7:

TABLE 7

Treatment B (according to the invention)
Free Fatty Acids in the Serum [.mu.mol/l],
MCT/vegetable oil/fish oil (50:40:10)

Subject	after	Day 1	Day 2	Day 3
1.	0 h	18	0	28
	5 h	1321	1421	1102
2.	0 h	298	254	431
	5 h	1252	1101	1038
3.	0 h	7	14	26
	5 h	1363	1286	1239
4.	0 h	25	8	7
	5 h	1179	1197	1095
5.	0 h	0	11	30
	5 h	1165	1502	1381
6.	0 h	4	0	19
	5 h	1556	1295	1417
7.	0 h	70	88	75
	5 h	1053	983	963
8.	0 h	0	12	0
	5 h	1421	941	1012
Average .+-. st.d.	0 h	53 .+-. 95	48 .+-. 82	77 .+-. 135
	5 h	1289 .+-. 150	1216 .+-. 187	1156 .+-. 160

TABLE 6

Treatment A (Comparative Example 1)
Free Fatty Acids in the Serum [.mu.gmol/l], MCT/vegetable oil (50:50)

Subject	after	Day 1	Day 2	Day 3
1.	0 h	0	22	39
	5 h	921	921	1068
2.	0 h	399	202	143
	5 h	996	742	762
3.	0 h	57	48	48
	5 h	1554	144	1408
4.	0 h	52	71	44
	5 h	1212	1173	979
5.	0 h	20	23	10
	5 h	903	1272	1405
6.	0 h	28	41	82
	5 h	1082	1271	1449
7.	0 h	97	90	122
	5 h	1068	949	1169
8.	0 h	27	47	34
	5 h	1219	1236	1140
Average .+-. st.d.	0 h	85 .+-. 122	68 .+-. 55	65 .+-. 43
	5 h	1119 .+-. 198	1126 .+-. 218	1173 .+-. 225

TABLE 8

Treatment C (Comparative Example 2)
Free Fatty Acids in the Serum [.mu.mol/l], MCT/vegetable oil/fish oil (50:30/20)

Subject	after	Day 1	Day 2	Day 3
1.	0 h	13	12	0
	5 h	1051	828	863
2.	0 h	271	67	82
	5 h	900	816	899
3.	0 h	0	20	1
	5 h	1010	941	1006
4.	0 h	32	136	128
	5 h	1175	1269	1229
5.	0 h	0	10	0
	5 h	1139	1159	1024
6.	0 h	15	34	21
	5 h	887	1252	1239
7.	0 h	180	283	177
	5 h	1340	1335	1135
8.	0 h	0	0	0
	5 h	873	811	852
Average .+-. st.d.	0 h	64 .+-. 97	70 .+-. 90	51 .+-. 65
	5 h	1047 .+-. 154	1051 .+-. 211	1031 .+-. 146

3. Determination of Eicosapentaenoic Acid (EPA, 20:5 n-3) Incorporation in Membrane Phospholipids of Platelets (Thrombocytes) and Leucocytes

The determination of the proportion of eicosapentaenoic acid in the membrane phospholipids of the thrombocytes and leucocytes of the eight subjects was performed by gas chromatography via the fatty acid methyl esters (percent by area method).

TABLE 9

Treatment B (according to the invention)
Eicosapentaenoic in thrombocytes and leucocytes, MCT/vegetable oil/fish oil (50:40:10)

	Day 1 (0 h)	Day 2 (0 h)	Day 3 (0 h)
EPA in thrombocytes	0.2 .+-. 0.1	0.7 .+-. 0.1	1.2 .+-. 0.1
Average .+-. st.d. (% by area)			
EPA in leucocytes	0.4 .+-. 0.1	0.7 .+-. 0.3	1.0 .+-. 0.3
Average .+-. st.d. (% by area)			

TABLE 10

Treatment C (Comparative Example 2)
Eicosapentaenoic in thrombocytes and leucocytes, MCT/vegetable oil/fish oil (50:30:20)

	Day 1 (0 h)	Day 2 (0 h)	Day 3 (0 h)
EPA in thrombocytes	0.4 .+-. 0.1	1.0 .+-. 0.1	1.7 .+-. 0.1

Average .+-. st.d. (% by area)		
EPA in leucocytes	0.4 .+-. 0.1	0.9 .+-. 0.1 1.4 .+-. 0.1
Average .+-. st.d. (% by area)		

A comparison of the results of table 9 with those of table 10 shows that in treatment C, for example, an EPA contents of 0.9% by area was found in leucocytes on day 2. From the fish oil content in treatment B according to the invention being only half as high, an EPA content of 0.45% by area would be expected. Surprisingly, however, a significantly higher value was found, namely 0.7% by area. A similar result is obtained for day 3 as well as for thrombocytes on days 2 and 3.

EXAMPLE 2

(in vitro)

Apoprotein Uptake into the Emulsion Particles

Of great interest is the significantly lower enrichment (t-test, two-sided) of apoprotein C-I ($p<0.0001$) and apoprotein C-III ($p<0.0001$), which are both apoproteins that inhibit both, triglyceride hydrolysis and direct uptake of the emulsion particles into the target tissue (such as the liver), in the emulsion particles having a composition according to the invention (preparation example 4) will presumably result in a more thorough intravascular scavenging of lipids than with the other lipid emulsion examined (preparation example 5).

TABLE 11

Uptake of Apoproteins C-I and C-III in Emulsion Particles, (incubation: 3 h), MCT/vegetable oil/fish oil (50.40:10) vs. MCT/vegetable oil/fish oil (50:30:20)

	MCT/vegetable oil/fish oil (50:40:10) (Preparative Example 4)	MCT/vegetable oil/fish oil (50:30:20) (Preparative Example 5)
Apo C-I Uptake [.mu.g] (n = 4) Average .+-. st.d.	5.1 .+-. 0.51	23.4 .+-. 1.43
Apo C-III Uptake [.mu.g] (n = 4) Average .+-. st.d.	30.1 .+-. 2.67	54.7 .+-. 4.00

Lipid emulsions for parenteral administration will interact with endogeneous lipoproteins. During the infusion, the exogeneously supplied emulsion partly fuses with endogeneous LDL (low density lipoprotein; $d<1.006$ g/ml), a lipoprotein with a high content of apoprotein B (apo B). Thus, the apo B enrichment in the fused emulsion particles is indicative of the extent of fusion of exogeneously supplied emulsion with endogeneous LDL which has a relatively long plasma half life. Therefore, a high content of apo B in the fused emulsion particles must be considered indicative of prolonged residence time of the infused lipids. Conversely, a low apo B content means a short plasma half life, corresponding to a reduced residence time in the plasma.

Two lipid emulsions according to preparative examples 4 and 5 were incubated with human LDL in lipoprotein-poor plasma at 37.degree. C. for

4 hours, followed by a determination of the content of apoprotein B in the emulsion fraction.

TABLE 12

Apoprotein B Content in the Emulsion Particles, MCT/vegetable oil/fish oil (50:40:10) vs. MCT/vegetable oil/fish oil (50:30:20)	MCT/vegetable oil/fish oil (50:40:10) (Preparative Example 4)	MCT/vegetable oil/fish oil (50:30:20) (Preparative Example 5)
Apo B Content [mg/dl] Average .+-. st.d.	0.05 .+-. 0.05 (n = 6)	0.27 .+-. 0.21 (n = 7)

The emulsion particles having a composition according to the invention show an apo B enrichment which is more than five times lower than that of the other lipid emulsion examined, corresponding to a higher hydrolysis rate. The difference is significant (t-test, two-sided; $p<0.05$).

CLM What is claimed is:

1. An isotonic lipid emulsion for parenteral administration comprising lipid droplets, wherein each such droplet comprises medium-chain triglycerides, at least one vegetable oil comprising triglycerides which supply **omega-6-fatty acids**, and at least one fish oil comprising triglycerides which supply **omega-3-fatty acids** wherein said lipid emulsion comprises, based on the total lipid content of the emulsion: from 30% to 60% by weight of the medium-chain triglycerides; from 35% to 65% by weight of the vegetable oil(s); and from 5% to 20% by weight of the fish oil(s).

2. The lipid emulsion according to claim 1, wherein said medium-chain triglycerides comprise at least 90% triglycerides of caprylic acid (C.sub.8) and capric acid (C.sub.10).

3. The lipid emulsion according to claim 1, wherein said vegetable oil is selected from the group consisting of safflower oil and soybean oil.

4. The lipid emulsion according to claim 1, wherein said fish oil is selected from the group consisting of sardine, salmon, herring, mackerel and other cold water fish oils and fish oils synthetically obtainable by re-esterification of glycerol with **omega-3-fatty acids** obtained by hydrolysis of cold water fish oil.

5. The lipid emulsion according to claim 1, wherein said fish oil contains at least 25% of eicosapentaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

6. The lipid emulsion according to claim 1, wherein said fish oil contains at least 12% of docosahexaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

7. The lipid emulsion according to claim 1, wherein the total lipid content is from 5% to 30% by weight, based on the weight of the emulsion.

8. A method for treating exaggerated **inflammatory** reactions, increased risk of vascular thrombosis or severe cardiac arrythmia by parenteral administration of the emulsion of claim 1 to a patient having an exaggerated **inflammatory** reaction, or an increased risk of vascular thrombosis, or severe cardiac arrythmia.

9. The lipid emulsion according to claim 1, wherein the average size of the said lipid droplets is less than 1.0 .mu.m.

10. The lipid emulsion according to claim 2, wherein said vegetable oil is selected from the group consisting of safflower oil and soybean oil.

11. The lipid emulsion according to claim 2, wherein said fish oil is selected from the group consisting of sardine, salmon, herring, mackerel and other cold water fish oils and fish oils synthetically obtainable by re-esterification of glycerol with **omega-3-fatty acids** obtained by hydrolysis of cold water fish oil.

12. The lipid emulsion according to claim 3, wherein said fish oil is selected from the group consisting of sardine, salmon, herring, mackerel and other cold water fish oils and fish oils synthetically obtainable by re-esterification of glycerol with **omega-3-fatty acids** obtained by hydrolysis of cold water fish oil.

13. The lipid emulsion according to claim 2, wherein said fish oil contains at least 25% of eicosapentaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

14. The lipid emulsion according to claim 3, wherein said fish oil contains at least 25% of eicosapentaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

15. The lipid emulsion according to claim 4, wherein said fish oil contains at least 25% of eicosapentaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

16. The lipid emulsion according to claim 2, wherein said fish oil contains at least 12% of docosahexaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

17. The lipid emulsion according to claim 3, wherein said fish oil contains at least 12% of docosahexaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

18. The lipid emulsion according to claim 4, wherein said fish oil contains at least 12% of docosahexaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

19. The lipid emulsion according to claim 5, wherein said fish oil contains at least 12% of docosahexaenoic acid in said triglycerides, based on the fatty acid methyl esters of the fish oil concentrate.

20. The lipid emulsion according to claim 2, wherein the total lipid content is from 5% to 30% by weight, based on the weight of the emulsion.

21. The lipid emulsion according to claim 3, wherein the total lipid content is from 5% to 30% by weight, based on the weight of the

emulsion.

22. The lipid emulsion according to claim 4, wherein the total lipid content is from 5% to 30% by weight, based on the weight of the emulsion.

23. The lipid emulsion according to claim 5, wherein the total lipid content is from 5% to 30% by weight, based on the weight of the emulsion.

24. The lipid emulsion according to claim 6, wherein the total lipid content is from 5% to 30% by weight, based on the weight of the emulsion.

25. A method for treating exaggerated **inflammatory** reactions, increased risk of vascular thrombosis, or severe cardiac arrythmia by parenteral administration of an emulsion provided in accordance with claim 2 to a patient having an exaggerated **inflammatory** reaction, or an increased risk of vascular thrombosis, or severe cardiac arrythmia.

INCL INCLM: 514/560.000
INCLS: 514/943.000
NCL NCLM: 514/560.000
NCLS: 514/943.000
IC [6]
ICM: A61K031-19
EXF 514/546; 514/560; 514/937; 514/943
ARTU 167
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 2 OF 33 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
AN 1998:58828 CAPLUS
DN 128:132421
TI Pharmaceutical compositions of spirulina algae and omega fatty acids for treatment of **inflammation** and pain
IN Bockow, Barry I.
PA USA
SO U.S., 6 pp.
CODEN: USXXAM
DT Patent
LA English
IC ICM A61K035-80
ICS A61K031-20; A61K031-60; A61K031-19
NCL 424093700
CC 63-6 (Pharmaceuticals)
Section cross-reference(s): 1
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5709855	A	19980120	US 1995-538992	19950922 <--

AB A compn. for preventing or treating **inflammation** and/or pain by topical administration is disclosed. The compn. contains an omega fatty acid in combination with spirulina. Preferably, the omega fatty acid is a mixt. of **omega-3 fatty acids** and **omega-6 fatty acids**. **Omega-3 fatty acids** include eicosapentaenoic acid (I) and docosahexaenoic acid (II), and **omega-6 fatty acids** include gamma-linolenic (III) acid and dihomo-gamma-**linolenic acid** (IV). The compn. may further include pharmaceutically acceptable carriers or diluents, vitamins A and E, and a cyclooxygenase inhibitor such as Me salicylate. A topical pharmaceutical contained I 0.1-20, II 0.1-15, III and/or IV 0.1-20,

spirulina 0.1-7, Me salicylate 3-25, vitamin A 0.5-3, vitamin E 0.5-3, squalene 5-20, Carbomer 2001 (2% soln.) 5-15, aloe vera 0.2-5, and water and other inert ingredients 30-60%. Patients suffering from different **inflammatory** conditions were treated for a period of 6-9 mo with the above compn. About 88% of the patients showed significant and sustained pain relieve along with improve quality of daily living.

ST pharmaceutical spirulina algae omega fatty acid; **inflammation** pain inhibition omega fatty acid; topical pharmaceutical eicosapentaenoate docosahexaenoate linolenate **inflammation**

IT Tendon
(disease, tendinitis; pharmaceutical compns. of spirulina algae and omega fatty acids for treatment of **inflammation** and pain)

IT Muscle diseases
(fibromyalgia; pharmaceutical compns. of spirulina algae and omega fatty acids for treatment of **inflammation** and pain)

IT Muscle diseases
(myositis; pharmaceutical compns. of spirulina algae and omega fatty acids for treatment of **inflammation** and pain)

IT Analgesics
Anti-**inflammatory** drugs
Antiarthritics
Antirheumatic drugs
Autoimmune diseases
Chronic fatigue syndrome
Osteoarthritis
Spirulina
Topical drug delivery systems
(pharmaceutical compns. of spirulina algae and omega fatty acids for treatment of **inflammation** and pain)

IT **Omega-3 fatty acids**
Omega-6 fatty acids
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(pharmaceutical compns. of spirulina algae and omega fatty acids for treatment of **inflammation** and pain)

IT **Inflammation**
(tendinitis; pharmaceutical compns. of spirulina algae and omega fatty acids for treatment of **inflammation** and pain)

IT 39391-18-9, Cyclooxygenase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(inhibitor; pharmaceutical compns. of spirulina algae and omega fatty acids for treatment of **inflammation** and pain)

IT 50-78-2, Acetylsalicylic acid 53-86-1, Indomethacin 69-72-7, biological studies 111-02-4, Squalene. 119-36-8, Methyl salicylate. 506-26-3, .gamma.-**Linolenic acid** 552-94-3, Salicylsalicylic acid 644-62-2 1406-18-4, Vitamin e 1783-84-2, Dihomo-.gamma.-**linolenic acid**. 6217-54-5, Docosahexaenoic acid 10417-94-4, Eicosapentaenoic acid 11103-57-4, Vitamin A 15307-86-5, Diclofenac 15687-27-1, Ibuprofen 22071-15-4, Ketoprofen 22204-53-1, Naproxen 22494-42-4, Diflunisal 26171-23-3, Tolmetin 29679-58-1, Fenoprofen 36322-90-4, Piroxicam 38194-50-2, Sulindac 41340-25-4, Etodolac 42924-53-8, Nabumetone 64425-90-7, Trilisate, biological studies 74103-06-3, Ketorolac
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(pharmaceutical compns. of spirulina algae and omega fatty acids for treatment of **inflammation** and pain)

L13 ANSWER 3 OF 33 USPATFULL
AN 1998:64731 USPATFULL
TI Anti-**inflammatory** and infection protective effects of sesamin-based lignans
IN Forse, R. Armour, Brookline, MA, United States
Chavali, Sambasiva, Boston, MA, United States
PA Beth Israel Deaconess Medical Center, Inc., Boston, MA, United States

(U.S. corporation)
PI US 5762935 19980609 <--
AI US 1995-429014 19950426 (8)
RLI Continuation of Ser. No. US 1994-201682, filed on 25 Feb 1994
DT Utility
FS Granted

REP	US 3901875	Aug 1975	424/195.100	Park
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EXNAM Primary Examiner: Kight, John; Assistant Examiner: Lee, Howard C.
LREP Lahive & Cockfield, LLP
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN No Drawings

AB The uses of lignans of the sesamin family to treat infection and **inflammation** is disclosed. These lignans may be delivered enterally or parenterally and either in the form of sesame oil or in purified form. A total parenteral nutrition solution or dietary supplement are the preferred forms of administration.

PARN This application is a continuation of application Ser. No. 08/201,682 filed on Feb. 25, 1994 Entitled: ANTI-**INFLAMMATORY** AND INFECTION PROTECTIVE EFFECTS OF SESAMIN-BASED LIGNANS. The contents of all of the aforementioned applications are expressly incorporated by reference.

SUMM BACKGROUND OF THE INVENTION

The present invention relates to the formulation and use of dietary supplements and nutritional solutions for enteral and parenteral treatment of the effects of infection. These same dietary supplements or nutritional solutions may also be used as anti-**inflammatory** agents. The active ingredient in the dietary supplement or nutritional solution is a lignan of the sesamin family. This same active ingredient has particular effectiveness in total parenteral nutrition solutions to provide similar benefits.

The last decade has seen an explosion in the exploration of the interaction between diet and disease. In particular, the effects of various amino acids and lipids in the diet on a variety of conditions including heart disease, hypercatabolic states, liver disease, immunosupresssion, and infection treatment have been uncovered. Often, the effects are far removed from the norm and as such are unexpected. One of the most important developments of this type has been the discovery that by changing the dietary lipid content, positive effects in health treatment beyond plasma fat modification could be achieved. While the early work in modifying lipid content and type in diet came from an understanding that saturated fats cause particular problems in heart disease, later work determined that not just the use of

polyunsaturated fats but also the type of polyunsaturated fat was important.

There are three major families of polyunsaturated fatty acids: .omega.3, .omega.6 and .omega.9. The names are based on location of the closest double bonds to the methyl end of the fatty acid; that is, if the closest double bond is between the third and fourth carbon atoms from the methyl group, the molecule is classified as an **.omega.3 fatty acid** while if the double bond is between the 6th and 7th carbon atoms, it is classified as an **.omega.6 fatty acid**. Mammals can desaturate or elongate fatty acid chains but cannot interconvert fatty acids from one family to another. The most important dietary fatty acids are the C.sub.18 and C.sub.20 fatty acids, primarily linoleic (C18:2.omega.6), **linolenic acid** (C18:3.omega.3) and dihomo-.gamma.- **linolenic acid** (C18:3.omega.6). Manipulation of the content of these fatty acids changes the arachidonic eicosapentanoic and docosahexanoic acid (C20:4.omega.6, C20:5.omega.3, and C22:6.omega. receptively) ratios and can cause far reaching effects in terms of immunosuppression, response to hypercatabolic states, and infection. For example, U.S. Pat. No. 4,752,618, issued Jun. 21, 1988, on an application of Mascioli et al., the disclosure of which is incorporated herein by reference, discloses the beneficial effects of **.omega.3 fatty acids** in the treatment of infection. In U.S. Pat. No. 5,260,336, issued Nov. 3, 1993, on an application of Forse et al., the disclosure of which is also incorporated herein by reference, concerns a method of minimizing the effect of catabolic illness or infection using an oil such as oleic acid which is rich in .omega.9 fatty acids. Other similar patents and articles, such as U.S. Pat. No. 4,810,726, issued Mar. 7, 1989, on an application of Bistrian et al., the disclosure of which is also incorporated herein by reference, disclose other means of treating illness using fatty acid dietary manipulation.

The "culprit" in many diets appears to be the high level of **.omega.6 fatty acids**, primarily linoleic acid, a precursor for the formation of arachidonic acid which is a substrate for the production of proinflamatory dienoic eicosanoids including PGE.sub.2 and TxA.sub.2 which can lead to elevated levels of thromboxane A.sub.2 and related prostanoids. Elevation of these prostanoids has been linked to problems in response to endotoxin challenge and other infection states. Accordingly, the new wave in diets has been to minimize the **.omega.6 fatty acid** content (which, although an essential fatty acid, is not needed in the quantities found in most commercial oils) while maximizing the **.omega.3 fatty acids** (e.g., fish oil) and .omega.9 fatty acids (e.g., canola oil).

One byproduct of the recent exploration of the relationship between dietary modification and health has been a renewed look at traditional homeopathic remedies. One of these is sesame oil, which has long been known as a traditional health or medicinal food. Recent studies of sesame oil, which contains primarily **.omega.6 fatty acids**, indicate that the health benefits from use of sesame oil is based not on the fatty acid content, but rather on a lignan included therein, sesamin. In fact, sesamin is but one of several related lignans found in sesame oil. These lignans include sesaminol, sesamolin, episesamin and episesaminol. A recent article entitled "Sesamin: A Multifunctional Gift From Nature", by M. Sugano and

K. Akimoto, Journal of Chinese Nutrition Society 18, 1-11 (1993), is a summary of known and projected effects of sesamin. This article suggests that the possible benefit of sesamin arise from its interference with linoleic acid metabolism, hypothesizing that the methodology is with interference with .delta.-5-desaturate, an enzyme that catabolizes the reaction from dihomo-.gamma.-**linolenic acid** (DGLA) to arachidonic acid (AA), an important step in linoleic acid metabolism.

The article also cites other papers discussing other possible beneficial effects of sesamin including hypocholesterolemic action, enhancement of hepatic detoxification of chemicals and alcohol, a protective effect against chemically induced mammary cancer, in vivo antioxidative action and more problematic, a potential link to immunopotentiation.

Although this list of possible beneficial effects of sesamin and its related lignans is impressive, nothing has been said or discussed on its possible effects on infection and/or **inflammation**. In fact, if the mechanism of action hypothesized is correct, i.e., affecting .delta.-5-desaturase, feedback inhibition might turn off this anti-infective activity and there would be no basis for any **inflammatory** activity. As is disclosed herein, there is now reason to believe that this proposed mode of operation is incorrect, and that the actual means of activity of sesamin and its related lignans is on either phospholipase A.sub.2 or cyclooxygenase. Either of these mechanisms could cause anti-**inflammatory** effects. It should be noted, however, that identifying the mode of operation is not required to practice the invention.

Accordingly, an object of the invention is to treat infection and those at risk with infection with a dietary supplement or nutrition solution which provides added health benefits.

A further object of the invention is to provide a dietary supplement or nutrition solution, e.g., a parenteral nutrition solution, which provides anti-**inflammatory** activity.

Another object of the invention is to provide a parenteral nutrition solution, preferably a total parenteral nutrition solution, which has both anti-**inflammatory** and anti-infection characteristics.

These and other objects and features of the invention will be apparent from the following description and the claims.

SUMMARY OF THE INVENTION

The present invention features a method of treating infection and **inflammation** as well as dietary supplements and parenteral nutrition solutions useful in the methods of the invention. These dietary supplements and parenteral nutrition solutions have lignans in the sesamin family as the active ingredient.

More particularly, the present invention concerns a method of treating infection and minimizing the possibility of infection in at risk persons by administering an effective amount of a lignan selected from the group consisting of sesamin, episesamin, sesaminol, sesamolin, episesaminol, and mixtures thereof. An "effective amount", as used herein, means an amount sufficient to show statistically significant anti-infection or anti-**inflammatory** effects. The range of effective amount is about 1-10 mg/kg body weight. These lignans can be administered in purified form, such as purified sesamin, or administered in the form of

sesame oil. For certain uses, enteral administration in the form of a dietary supplement containing an effective amount of the lignan is preferred, while for others, parenteral administration may be preferred.

The dietary supplement should include essential fatty acids and, possibly, essential vitamins and minerals in addition to lignan. In its most fulsome form, the parenteral nutrition solution may be used as a total parenteral solution, containing all essential nutrients for health. These same solutions may be used not just for treating infection but also for treating **inflammation**.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the beneficial effects of sesamin and its related lignans on treatment of infection and/or **inflammation**. As noted, the common hypothesis for all actions of sesamin described to date has been the theory that it affects the enzyme .delta.-5-desaturase. In contrast, it appears that this scientific theory may be wrong and that it appears instead to be that sesamin inhibits the activity of cyclooxygenase (an enzyme which converts arachidonic acid to its metabolites) or the activity of phospholipase A.sub.2 (an enzyme which releases arachidonic acid from membrane phospholipids). As such, since drugs which inhibit the activity of phospholipase A.sub.2 (such as aspirin and several steroids) or cyclooxygenase (such as indomethacin) have anti-**inflammatory** effects, it appears that sesamin and its related lignans could be used as anti-**inflammatory** agents. Further, as will be shown herein, direct testing shows that sesamin has surprising anti-infection capabilities.

The following non-limiting examples show the activity of these lignans in terms of lipid metabolism and infection treatment.

DETD EXAMPLE 1

In this example, the effects of a sesame oil diet on circulating lipids and the mode of activity of the lignans is investigated.

More particularly, if, as has been postulated by others, sesamin inhibits .delta.-5-desaturase activity, it would be expected that a decrease in arachidonic acid levels would coincide with an accumulation of dihomo-.gamma.-linoleic acid from the sesame oil diet. However, under this mode of operation, there should be no effect on PGE.sub.2 or TxB.sub.2 levels. In contrast, if the PGE.sub.2 and TxB.sub.2 values are modified, this would not support the .delta.-5-desaturase mode of activity but rather a cyclooxygenase or phospholipase A.sub.2 activity mode.

In this and the following example, a comparison was made between two diets which were as close in fatty acid and nutritional content as possible except one contained sesame oil, and its associated sesamin lignans, while the other was based on safflower oil. Sesame oil (Welch, Holme & Clark Company, Inc., Newark, N.J.), and safflower oil (SVO Specialty Products, Culberton, Mont.), provided the .omega.6 fats. Palm oil and Trisum (high oleic sunflower oil) were used as fat fillers. Table 1 shows the fat portion of the diet.

TABLE 1

Safflower Oil	Sesame Oil	Palm Oil	Trisum

SO	52	g	0		88	g	10	g
SSO	34	g	34	g	82	g	0	

The lipid portions of each diet were approximately equal in the amounts of saturated, monounsaturated and polyunsaturated fats (approximately 10% each) and also equal in the amount of linoleic acid.

One hundred fifty grams of the lipid was added to 850 g. of AIN-76 basel diet, a fat-free basel diet which contains essential minerals and vitamins. The diets each had 30% of the calorie value and 15% by weight formed from the oil. An antioxidant, t-butyl hydroxytoluene (0.05%) was added and the resulting diets were thoroughly mixed. The diets were prepared in bulk, partitioned into daily rations, and stored at 4.degree. C.

Balb/c mice (Jackson Laboratories) were fed the diets ad libium for a period of three weeks. The animals were fed every day before dusk. The phospholipid fatty acid compositions of plasma and of the liver cell membranes were determined by gas chromatography following thin layer of chromatography. The results showed a 1-3% incorporation of DGLA into the phospholipids from both the plasma and liver cell membranes for those mice fed with the sesame oil diets while none was found in those fed with the safflower oil diets. In addition, ten animals of each group were injected with 10.mu. g/kg body weight lypopolysacchride which induces the production of proinflamatory mediators. Plasma samples were collected 90 minutes later, stored at -90.degree. C., and used to determine if there were any effects on the concentration of the pro-**inflammatory** mediators, TNF-.alpha., PGE .sub.2 and TxB.sub.2. These mediators decreased by approximately by 50% in the sesame oil diets when compared with the safflower oil diet.

The experimental determination of a marked decrease in PGE.sub.2 and TxB.sub.2 after sesame oil diet show what would be expected if cyclooxygenase or phospholipase A.sub.2 were the affected enzymes. Thus, it appears that the mode of operation suggested by the prior articles is probably incorrect and the modes of operation proposed herein are correct. In addition, since the modes of operation now postulated (and confirmed by experiment) are the same as are shown for a variety of steroidal and other anti-**inflammatory** drugs such as aspirin or indomecithin, these lignans should have similar anti-**inflammatory** properties.

EXAMPLE 2

In this example, the same diets and mice were used to determine if diet modification had any effect on the ability of the animals to withstand infection. The animals were fed the diets for three weeks ad libium.

At the end of the three week feeding period, twenty animals in each group underwent cecal ligation and puncture. The mice were anaesthetized and then shaved over the anterior abdominal wall. A midline incision approximately 2 cm long was made, sufficient to expose the cecum and adjoining intestine. With a 3-0 silk suture, the cecum was tightly ligated at its base without causing bowel obstruction. The cecum was then punctured twice with a 22 gauge needle, gently squeezed to exude feces and to ensure that the two puncture holes did not close. The overlapping abdominal incision was then closed and 1 ml of saline was administered subcutaneously for fluid resuscitation. This cecal ligation and puncture is a widely accepted form of infection model to resemble

abdominal sepsis. See, e.g., C. Baker et al., "Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model," Surgery(Aug. 1983), 331-335. Survival of the mice is the normal measure of treatment effectiveness.

Thirteen of the twenty mice in the group maintained on the sesame oil diet survived (65%) while only four of the twenty mice in the safflower oil diet survived (20%). Using a student t-test, the mortality rates were significantly different ($p<0.01$). Accordingly, it is clear that not only does a diet including sesamin reduce the levels of **inflammatory** molecules such as TNF-.alpha., PGE.sub.2 and TxB.sub.2 but it also provides protection against infection.

Those skilled in the art will recognize other alternative forms of the invention besides those disclosed in the above examples. There examples are merely exemplary of the invention which is defined by the following claims.

CLM What is claimed is:

1. A method of treating infection and minimizing the possibility of infection in at risk persons comprising administration of an effective amount of sesame oil to the at risk persons.

2. The method of claim 1 wherein said sesame oil is administered enterally.

3. The method of claim 2 wherein said enteral administration comprises administration of a dietary supplement containing an effective amount of said sesame oil.

4. The method of claim 3 wherein said dietary supplement further comprises essential vitamins and minerals.

5. The method of claim 1 wherein said sesame oil is administered parenterally.

6. The method of claim 5 wherein said parenteral administration comprises administration of said sesame oil as part of a total parenteral nutrition diet.

7. The method of claim 5 wherein said sesame oil is administered as part of an oil included in a parenteral diet.

8. A method for treating infection or protecting against infection associated with abdominal sepsis, comprising administering to an animal in need thereof an effective amount of sesame oil.

9. The method of claim 8, wherein said sesame oil is administered enterally.

10. The method of claim 8, wherein said enteral administration comprises administration of a dietary supplement containing an effective amount of said sesame oil.

11. The method of claim 10, wherein said dietary supplement further comprises essential vitamins and minerals.

12. The method of claim 8, wherein said sesame oil is administered parenterally.

13. The method of claim 8, wherein said parenteral administration

comprises administration of said sesame oil as part of a total parenteral nutrition diet.

INCL INCLM: 424/195.100
INCLS: 514/469.000; 514/783.000; 514/885.000
NCL NCLM: 424/776.000
NCLS: 424/725.000; 514/469.000; 514/783.000; 514/885.000
IC [6]
ICM: A61K035-78
EXF 426/804; 426/810; 514/464; 514/468; 514/783; 514/825; 514/886; 514/887; 514/904; 514/905; 424/195.1; 424/DIG.13
ARTU 121

L13 ANSWER 4 OF 33 USPATFULL
AN 1998:48451 USPATFULL
TI Use of **.omega.-3-fatty acids**
IN Egberg, Nils, Lidingo, Sweden
Larsson-Backstrom, Carin, Stockholm, Sweden
Jakobsson, Jan, Djursholm, Sweden
Lundh, Rolf, Huddinge, Sweden
PA Pharmacia & Upjohn Aktiebolag, Stockholm, Sweden (non-U.S. corporation)
PI US 5747533 19980505 <--
WO 9316691 19930902
AI US 1994-290905 19941021 (8)
WO 1993-SE146 19930223
19941021 PCT 371 date
19941021 PCT 102(e) date
PRAI SE 1992-541 19920224
DT Utility
FS Granted
REP US 4678808 Jul 1987 514/560.000 Ward et al.
US 4820731 Apr 1989 514/549.000 Mascioli et al.
US 4960795 Oct 1990 514/560.000 Salte et al.
US 5223285 Jun 1993 426/072.000 DeMichele et al.
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EXNAM Primary Examiner: Weddington, Kevin E.
LREP Pollock, Vande Sande & Priddy
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 5 Drawing Page(s)

AB The present invention relates to **omega-3-fatty acid** containing preparations for the treatment of Disseminated Intravascular Coagulation (DIC) and symptoms related to DIC, as well as such preparations for reducing a pathological increase in pulmonary artery pressure (PAP). The preparations may be in the form of emulsions, or aerosols for inhalation, of an oil or phospholipids or other derivatives or salts of **omega-3-fatty acids** of marine and/or vegetable origin with a significant content of **omega-3-fatty acids**. The preparations may also be in tablet or capsule form for oral use.

SUMM The present invention relates to the use of **omega-3-fatty acids** (hereafter called .**omega**.**3-fatty acids**) for the treatment of or for preventing the development of Disseminated Intravascular Coagulation (hereafter called DIC; for abbreviations, see the appended Abbreviation List) as well as reducing a pathological increase in pulmonary artery pressure (PAP). The preparations to be used may be in the form of emulsions for parenteral or enteral administration, or for example in the form of aerosols for inhalation or in a form for oral administration. The .omega.3-fatty adds (or salts or derivatives thereof) can originate from a marine or vegetable oil, from phospholipids, or be of syntheic origin.

BACKGROUND OF THE INVENTION

Today one of the major challenges for intensive care is to combat the secondary hypoperfusion syndromes seen after septicemia, trauma and malignancies. These syndromes include uncontrolled activation of the cascade systems (coagulation, fibrinolyses, kallikrein-kinin-, complement systems) often described as post traumatic micro embolism or disseminated intravascular coagulation (DIC). Disseminated intravascular coagulation (DIC) gives rise to a wide variety of symptoms, to some extent caused by massive disseminated microembolism. There seem to be various target organs partly due to the inducing agent or cause as well as to probably a number of unknown factors. A common situation in patients who develope DIC is pulmonary microembolism which could lead to severe problems of gas exchange, oedema and subsequent increase in

pulmonary arterial pressure (PAP).

According to the literature (Medicine, Edited by E. Rubenstein and D. D. Federman published by Scientific American, New York, 1988, chapter 5:VI, Hemostasis and coagulation, p35-38) there are a plurality of circumstances that can initiate the DIC-syndrome. Such circumstances may be massive tissue damage, leading to the release of huge amounts of tissue thromboplastic materials, causing extensive activation of the extrinsic system or extensive destruction of endothelial surfaces. The circumstances can be caused by for example severe injuries and infections, tumor products, hemolytic transfusion reactions, vasculitis, heatstroke, hemangomias and certain snake bites. In all cases this leads to a massive activation of the hemostatic mechanisms, which overwhelms the inhibitor mechanisms.

The entire scheme of coagulation either initiated by the intrinsic, including the kallikrein-Factor XII, pathway or extrinsic pathways is finely tuned to culminate in a burst of thrombin activity, causing hemostatic activity at the site of the injury, which leads to deposition of cross-linked fibrin to form a hemostatic plug. Normally, the effects of intravascular coagulation are controlled or modulated by the dilutional effects of the blood flow, by antithrombines, antiplasmin and among other factors the mechanisms that down-regulate hemostasis. However, these control mechanisms can be overwhelmed and disordered by the circumstances mentioned above. This may lead to excessive release of thrombin, which results in thromboses, ischemic conditions and necrosis.

The DIC syndrome can thus lead to massive intravascular deposition of fibrin and impaired nutritive circulation leading to organ failure. This picture of DIC has for a long time dominated the concept of this common disorder. However, alternative views on this syndrome has also been brought forward. The fact that probably several other enzyme systems are involved in the syndrome has been focused on by suggestions on alternate names like "defibrination syndrome". This is discussed in the article of G. Muller-Berghaus in Seminars In Thrombosis and Hemostasis, vol. 15, No. 1, 1989, page 58-87, which is referred to for a review of the numerous conditions related to DIC.

Despite modern treatment modalities, the high mortality rate (>50%) from DIC has not decreased appreciably over the last 20 years. A great number of various therapies have been tried in order to prevent and also treat this syndrome. The treatment of DIC has for a long time been focused on an inhibition of the coagulation process by means of administration of heparin, antithrombin concentrates or hirudin. Depending on the activation mechanism(s) and the dominating symptoms alternative treatments have been suggested like dextran, acetylsalicylic acid, aprotinin, tranexamic acid and even streptokinase. Today corticoid steroid treatment is one of the pharmacological interventions that are frequently tried on these syndromes (C. Putterman, J Critical Care 5(4), 241-251, 1990). Until now however, there has been no consensus how to

combat these syndromes. Furthermore there are no scientific proofs for any specific regim in order to prevent this syndrome. Aggressive fracture stabilisation, optimal pain relief and adequate antibiotic therapy is of course of vital importance.

A most interesting report by C. Esmon and co-workers (Thrombos Haemostas 66(1), 160-165, 1991) demonstrated the effect of Protein C.sub.a on an experimental animal model where a disseminated intravascular coagulation was induced by infusion of endotoxin from E. coli bacteria. It was shown, that unlike heparin, which only prevented the fibrin formation but not the shock, Protein C.sub.a prevented the development of the whole syndrome. It was further suggested that the dramatic effect of Protein C.sub.a was probably due to a combined inhibitory effect on hemostasis and on the **inflammatory** reactions included in the endotoxin induced syndrome.

Another approach for the treatment of DIC has essentially aimed at a substitution of consumed coagulation factors and inhibitors. Substitution has been given as full plasma or plasma concentrates of coagulation factors or antithrombin. An adequate treatment of the underlying disease or trauma as well as good general care for circulation and ventilation, has on the other hand been shown to be the most effective way to eradicate this dangerous complication.

Different fatty acids in the lipids have different physiological, biochemical and pharmacological properties and during the last years great interest has been concentrated on the importance of the polyunsaturated **.omega.3-fatty acids**, containing 18-22 carbon atoms. The **.omega.3-fatty acids** eicosapentaenoic acid (20:5 .omega.3, EPA) and docosahexaenoic acid (22:6 .omega.3, DHA) are essential fatty acids in man. Besides their nutritional value, they are also known to possess pharmacological effects. The best known are the cardiovascular effects, the beneficial effects on **inflammatory** and autoimmune diseases and the necessity of these fatty acids for the normal development of brain and retina functions. These effects have such an importance that a lot of work has been done to find good nutritional compositions containing a high amount of (.omega.3-fatty adds. See e g WO 87/02247 (Baxter) and U.S. Pat. No. 4,820,731 (New England Deaconess Hospital) in which marine oils are used which contain a high amount of the **.omega.3-fatty acids** EPA and DHA. Early observations by Dyerberg et al. (Lancet, ii;117-119, 1978) indicated that there was an association between a high intake of **.omega.3-fatty acids** and prolonged bleeding time in Greenland Eskimos. One explanation for the prolonged bleeding time was further shown to be a suppression of the thromboxane A.sub.2 (TxA.sub.2) synthesis leading to an impaired platelet function. When fish oils are ingested and EPA displaces arachidonic acid (AA), precursor for eicosanoides, from cell membrane phospholipids, eicosanoides from a different series, 3-series, are produced. The thromboxane formed from EPA, unlike that from AA, has very little physiologic activity, whereas the prostacyclin is fully active, leading to an increased total antithrombotic and antiatherogenic prostacyclin activity (Leaf & Weber, n-3 News vol III (4), 1988). However, the **.omega.3-fatty acids** in fish oil may influence blood clotting, thromboses and fibrinolysis in many ways.

Tissue plasminogen activator (t-PA) is released from vascular endothelial cells after various kinds of stimuli. Plasminogen is activated by t-PA to plasmin which is the fibrinolytically active enzyme. The thrombolytically active t-PA can cause a dissolution of dots

within blood vessels and, thus, prove useful in acute treatment of heart attacks. Dietary supplements for weeks of fish oil has been shown to increase endogenous t-PA production (Barcelli et al, Thromb Res, 39, 307-312, 1985). This action should be the major deterrent to the development of blood clots in coronary arteries which are usually the terminal events blocking blood flow to the heart muscle thus causing heart attacks.

An elevated level of plasma fibrinogen has been identified as a risk factor for coronary artery disease. Dietary intake of fish oil for weeks suppresses the fibrinogen level (Hostmark et al, Br Med J (Clin Res) 297 (6642), 180-181, 1988). Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are both involved in the development of an **inflammatory** response. Dietary intake of fish oil for weeks has been shown to reduce the production of IL-1 and TNF (Endres et al, Clin Immunol Immunopath, 49, 424-438,1989).

The endothelial derived relaxing factor (EDRF), recently shown to be identical to nitrogen oxide (NO), has a relaxing effect on vascular smooth muscle and counteracts agents causing vasoconstriction resulting in hypoxic vascular damage. NO has also an antithrombotic and cytotoxic action. The cytotoxicity of activated macrophages against tumor target cells was shown to be dependent on the presence of NO (for references see Moncada et al., Pharmacological Reviews, vol.43, No 2, 1991). Fish oil feeding for weeks is claimed to enhance the effect of EDRF (Vanhoutte et al, In: Health effects of .omega.3 polyunsaturated fatty acids in seafoods. Eds Simopoulos et al, Karger, 233-244, 1991).

Tissue factor (TF), earlier referred to as tissue thromboplastin, is a potent trigger of the extrinsic pathway of blood coagulation. TF is produced by a large number of cell types, though not endothelium. Monocytes can be stimulated by lipopolysaccharides (LPS), which are toxic bacterial material, to expression of TF. Liposomes prepared from soybean lecithin can enhance the LPS effect of inducing thromboplastin in monocytes in the blood. TF expression, as induced by LPS and liposomes, was reduced by 40% after 8 weeks of fish oil diet (Osterud et al, Omega-3 News vol V (2),1990). The mechanism for this inhibition probably includes an inhibition of arachidonic acid metabolism to eicosanoides, by cyclo-oxygenase to prostanoides and by lipoxygenase to leucotrienes (Osterud et al, Abb Med, 21, 47, 1989). Non-steroidal anti-**inflammatory** drugs (NSAID), like acetylsalicylic acid, however, block only the cyclo-oxygenase and thereby provide more substrate for the lipoxygenase pathway, leading to 50-250% enhanced monocyte activation as expressed by induced thromboplastin activity. Thus, to create an inhibition on **inflammatory** reactions as well as on blood coagulation, fish oil might be a better treatment than NSAID.

The above mentioned mechanisms are involved in reactions which may lead to the DIC syndrome.

In an animal experimental model we have previously studied a DIC-like syndrome induced by infusion of plasma kallikrein (Egberg et al, Fibrinolysis, 2, 95-100; 101-106, 1988). In the initial studies we followed the plasma concentrations of prekallikrein and free kallikrein activity as well as coagulation factor XII and fibrinogen. The major inhibitor of plasma kallikrein, C.sub.1 -esterase inhibitor, as well as antithrombin, .alpha..sub.1 -antitrypsin and .alpha..sub.2 -macroglobulin were also determined. Fibrinolytic variables like plasminogen and .alpha.2-antiplasmin were additionally followed. The conclusions drawn from these studies were that a slowly progressing DIC was induced leading to a small but progressive consumption of

coagulation factors. There was also a progressive consumption of .alpha.2-antiplasmin, indicating a comparatively intense activation of the fibrinolytic system. These findings lead to the conclusions that we should look closer for a possible platelet activation. This was done by following the urinary excretion of the major thromboxane A.sub.2 metabolite, 2,3dinor-tromboxane B.sub.2. Thromboxane A.sub.2 is synthetized by platelets after various types of platelet stimulation and is one of the most powerful platelet aggregating agents known. At the same time we followed the urinary excretion of the major metabolite of prostacydin, 2,3-dinor-prostaglandin F.sub.1 a. Prostacyclin is synthetized and released from the vascular endothelium and is possibly the most effective inhibitior of platelet aggregation in the body. We also decided to look at the activation of the fibrinolytic system by measuring the plasma level of tissue plasminogen activator, t-PA.

In the previous study on a DIC-like syndrome we found a rise of the urinary excretion of thromboxane and prostacyclin metabolites after the kallikrein injections, indicating that the arachidonic acid metabolism was stimulated. The increased excretion rate for thromboxane metabolites suggested an in vivo aggregation of platelets that could contribute to the syndrome developed. A marked fall of the leucocyte count, which may be explained by aggregation of leucocytes, was also found. We also found an increase of the plasma level of t-PA, which probably was the cause of the earlier observed signs of activation of the fibrinolytic system. Of the cardiovascular parameters measured pulmonary artery pressure (PAP) increased and blood pressure (BP) was reduced.

DESCRIPTION OF THE INVENTION

We have found surprisingly that preparations containing **.omega .3-fatty acids** have extraordinarly advantageous effects for the treatment of DIC including a reduced pathological increase in pulmonary artery pressure (PAP).

In the present investigation we have studied the changes in the hemostatic system in a DIC model induced by injection of plasma kallikrein after pretreating the animals with marine oil emulsion.

We claim the use of .omega.3-fatty adds for the preparation of a medicament to be useful in the treatment of, or for preventing the development of DIC or for reducing the pathologically increased PAP. The **.omega.3-fatty acids** may come from marine oils, vegetable oils rich in .omega.3-fatty adds or from phospholipids containing .cndot.3-fatty acids. The **.omega. 3-fatty-acids** may also be in the form of synthetic derivatives or salts thereof.

Suitable administration forms are emulsions for parenteral, peroral or oral use, where the emulsions may be of therapeutic value or adapted for TPN (Total Parenteral Nutrition). Other suitable administration forms are inhalable aerosols, dosage forms to be administered nasally and orally in the form of tablets and capsules. The preferred **.omega .3-fatty acids** are EPA and/or DHA or their salts or derivatives.

The beneficial effects of fish oil after oral administration are first obtained after weeks of treatment. In order to reduce the incidence of DIC, which is mostly an acute situation, the effects of fish oil should be obtained with a short onset of action. The **.omega.3 -fatty acids** ought therefore to be active in some

intravenously or inhalable active and tolerable administrative form.

In the present invention we have used .**omega**.3-**fatty acids** in a preparation derived from marine oils in the form of an oil in water emulsion as prepared and presented below in Example 1. It must be clearly stated that other preparations containing .**omega**.3-**fatty acids** or derivatives thereof must be considered to be useful in the treatment of DIC. Such preparations may be types of emulsions or solutions other than specified in Example 1, with more concentrated .**omega**.3-**fatty acids**, with appropriate diluents or carriers, as well as oral preparations containing the .**omega**.3-**fatty acids** as salts of glycerol- and ethyl esters, phospholipids or sterols or other derivatives of .**omega**.3-**fatty acids** and suitable excipients. The oral preparations may be in conventional tablet form or in capsules manufactured according to well known techniques.

As alternatives to the mentioned preparations aerosols can be effective, both in a conventional form and in a form where the .**omega**.3-**fatty acids** are comprised in eventually bilayer forming phospholipids (liposomes) and different nasal preparations. The aerosols are intended to be administered by inhalation to the lungs, but may also be adapted to be administered through the nasal mucous membranes.

Useful emulsions could comprise 0.5-50% (w/v of total emulsion) oil, preferably 5-30% (w/v), vegetable oils, such as soybean oil, coconut oil, cottonseed oil, safflower oil, sunflower seed oil, linseed oil, borage oil, blackcurrent seed oil, canola oil or other vegetable oils containing .**omega**.3-**fatty acids**, or marine oil, or a mixture of those mentioned. The amount of the phospholipids could be 0.1-80% (w/v of total emulsion), preferably 0.1-20% (w/v). The preparation should contain .**omega**.3-**fatty acids** or derivatives thereof to an amount of 0.5-100%.

Phospholipids such as egg yolk or soybean phospholipids, marine phospholipids or synthetic emulsifiers can also be included in the emulsion. The total amount of emulsifier is preferably 0.1-20% (w/v of total emulsion). The emulsion can also contain other components which are normally incorporated in emulsions e.g. monoglycerides of fatty acids, components for adjusting isotonic properties (such as glycerol), antioxidants such as .alpha.-tocopherol, components for adjusting stability such as amino acids, and carbohydrates such as fructose and glucose etc.

Antioxidants should be added to protect the unsaturated .**omega**.3-**fatty acids** from oxidation. Such antioxidants could be .alpha.-tocopherol (Vitamin E), Vitamin C, carotenoides or retinoides. However, other antioxidants can be used which are active to protect the unsaturated .**omega**.3-**fatty acids** from oxidation in the preparation, after administration and after incorporation into biological membranes. A study of antioxidants in marine oil emulsions has been performed and is presented in Example 2 below.

The preparation of the emulsion is carried out in a conventional manner. Thus the lipids are mixed with the aqueous phase, phospholipids and optionally other emulsifiers and auxiliary agents in a suitable mixing device. Then the blend is homogenized to a desired particle size, preferably less than 1 micron. The ways to adjust the emulsion to a suitable particle size is well known to a person skilled in the art.

DIC is frequently still seen in combination with major trauma, septicemia, meningoencephalitis and pancreatitis. There is often, however, a 12-26 hours elapse between the onset of symptoms and the debut of coagulation and respiratory distress. Major efforts are today spent on therapeutic manoeuvres in order to prevent these disorders from appearing, by early fracture stabilisation, antibiotics, pain management, stress reduction and the goal is also to optimize oxygen delivery. Still these syndromes appear in a non-systematic fashion and with a high frequency of most complicated course Therefore it seems most interesting, that some therapy could be given in advance to most patients at risk, with the potential effect of lowering the incidence of DIC.

We have now found that preparations containing **.omega.3-fatty acids** have advantageous effects for the prevention of or the treatment of DIC, so that the incidence of DIC will be reduced. The DIC syndrome may be a syndrome as such or included in other syndromes like pulmonary microembolization, multiple organ failure (MOF), sepsis and other infectious and ischemic conditions. The effects of .omega.3-fatty adds are seen very early, already after two hours of infusion, and can thus be of value for treatment and prevention of the development of the DIC syndrome and to reduce the incidence of DIC and related syndromes, which mostly are acute situations.

The preparations containing **.omega.3-fatty acids** will also be useful in the treatment of DIC-related symptoms and conditions, such as increased pulmonary arterial pressure (PAP) and those described in the articles in Seminars In Thrombosis And Hemostasis vol. 14, No. 4, 1988, pages 299-338 (RL Bick) and in Seminars In Thrombosis And Hemostasis vol. 15, No. 1, 1989, pages 58-87 (G Muller-Berghaus).

The **.omega.3-fatty acids** in fish oil may influence on blood clotting, thrombosis and fibrinolysis in many ways. The beneficial effects of fish oil is relevant for the treatment of the DIC syndrome. The **.omega.3-fatty acids** may reduce the hemostatic changes so that coagulation, fibrinolysis and thrombosis parameters are kept in balance. The end points of these effects are reduction of fibrin deposits, reduced microembolism and reduced tendency for increased pulmonary artery pressure and to prevent a decrease in PaO.sub.2. In subchronic/chronic cases of DIC (see Seminars In Thrombosis And Hemostasis, vol. 14, No. 4, 1988, R. L. Bick), of which some patients are treated with total parenteral nutrition (TPN), e.g. malignancies (gastrointestinal, pancreas, prostate, lung, breast), chronic **inflammatory** disorders (Chrons disease), the effects of **.omega.3-fatty acids** may be obtained in lower doses administered for a longer period of time. In more acute situations with DIC (see the mentioned article of R. L. Bick, 1988), e.g. bacteremia, burns, disseminated malignancy, liver disease, vascular disorders, the effects of fish oil should be elaborated rapidly enough to be of value to reduce the incidence of DIC.

In order to obtain an acute effect the **.omega.3-fatty acids** should be administered intravenously in the form of an emulsion. The acute effect should appear within hours and therefore the dose needed can be expected to be relatively high.

In the experiments performed and presented below in Example 4 it is shown an improvement of the DIC syndrome already after a two hour infusion of marine oil emulsion. The PAP and thus the ventilatory complications associated with DIC in the lungs were reduced, PaO.sub.2 was maintained, the tendency of the platelets to aggregate was minimized, the fibrinolytic response was increased and no fibrin deposits could be found in any of the organs tested (kidney, lung, heart and spleen). The reduced level of fibrinogen, seen after only two hours of infusion of marine oil emulsion, may together with other positive effects shown for **.omega.3-fatty acids**, minimize the vulnerability for heart attacks. The reduction in RBC viscosity indicates increased nutritional blood flow through the capillaries. The positive effects on hemostasis are obtained before, and with a lower dose than that needed to suppress the immune defence. The positive effects on hemostasis and DIC are obtained after a short single infusion, which together with the anti-**inflammatory** effects expected after repeated infusions may have wide implications on DIC related symptoms. This anti-**inflammatory** effect is in favour over that obtained with NSAID:s, since these drugs block only cyclo-oxygenase and thereby provides more substrate for the lipoxygenase pathway, leading to enhanced monocyte activation as expressed by increased lipoxygenase effects and induced tissue factor activity. A concomitant vasodilation and increase in nutritional blood flow, reduction in PAP and maintained PaO.sub.2 is a great and totally unexpected advantage compared to present treatment with vasodilators, which usually reduce PaO.sub.2. This, together with increased fibrinolysis, reduced thrombogenicity and fibrin deposits, and the rapid onset of action enables the long-chain polyunsaturated fatty acids a unique and unexpected possibility to treat and prevent the development of DIC.

Lipid emulsions or other preparations containing **.omega.3-fatty acids**, such as aerosols for inhalation, containing **.omega.3-fatty acids**, are useful therapeutically to treat severe trauma and to treate and help to prevent the development of various forms of DIC. Such emulsions are also nutritionally useful, for example to patients with DIC, who also need parenteral nutrition (TPN) for a shorter period, or in long term TPN to reduce the symptoms of more chronic forms of DIC.

The invention thus relates to the use of **.omega.3-fatty acids** or their derivatives in emulsions or in other preparations with therapeutic effects for various forms of DIC and DIC-related symptoms as increased PAP or to reduce the incidence of these symptoms and also, in combination with TPN, to these patients. The administration form can be by parenteral infusion or inhalation of aerosols containing **.omega.3-fatty acid** rich phospholipids or nasal preparations to thereby obtaining acute as well as chronic, long-lasting effects, or by peroral or oral administration in more chronic situations with DIC or in inhalations of liposomes as **.omega.3-fatty acid** containing phospholipids to reduce the risk of complications related to pulmonary microembolization. The doses of **.omega.3-fatty acids** to be administered in an acute situation (1-2 days) may be high in order to approach the level of the therapeutic window. For therapeutic use over a

longer time period with repeated administration the dose of .**omega.3-fatty acids** may be reduced to approach the amount of **.omega.3-fatty acids** which should be of not only therapeutic but also of nutritional value. For nutritional use in TPN the .omega.3-fatty adds should be administered together with other fatty acids.

DETD Various modifications and equivalents of the emulsion or other forms of therapeutic preparations will be apparent to one skilled in the art without departing from the spirit or scope of the invention. It is therefore to be understood that the invention is not to be limited to the specific examples and embodiments disclosed herein.

EXAMPLES

EXAMPLE 1

PREPARATION OF AN EMULSION CONTAINING FISH OIL AND EGG YOLK PHOSPHOLIPIDS

The emulsion contained:

Fish oil	200	g
Egg yolk phospholipids	12.0	g
Glycerol	22.2	g
Aq. ad inject.	750	g
NaOH, 1M	1.3	ml

As antioxidant vitamin E (.alpha.-tocopherol) was added to the emulsion in an amount stated in the respective example.

The ingredients above were mixed in a "Ultra Turrax" and thereafter homogenized in a "Moulin-Gaulin High Pressure Homogenizer" The fish oil used had the following fatty acid content in %:

14:0	Myristic acid	6.3
16:0	Palmitic acid	14.7
16:1 (.omega.7)	Palmitoleic acid	7.3
18:0	Stearic acid	2.6
18:1 (.omega.9)	Oleic acid	8.9
18:1 (.omega.7)	Vaccenic acid	3.1
18:2 (.omega.6)	Linoleic acid	1.1
18:3 (.omega.3)	**Linolenic acid**	0.7
18:4 (.omega.3)	Stearidonic acid	2.6
20:1 (.omega.9)	Eicosenoic acid	1.5
20:4 (.omega.6)	Arachidonic acid	1.4
20:5 (.omega.3)	EPA	17.8
22:1 (.omega.11)		

	Docosaenoic acid	2.2
22:5 (.omega.3)	Docosapentaenoic acid	2.9
22:6 (.omega.3)	DHA	13.5

Total amount of fatty adds: 100% (w/w).

The egg yolk phospholipids used had the following fatty acid content in % of total fatty adds (w/w):

14:0	Myristic acid	0.2
16:0	Palmitic acid	31.5
16:1 (.omega.7)	Palmitoleic acid	1.2
18:0	Stearic acid	14.1
18:1 (.omega.9)	Oleic acid	28.0
18:2 (.omega.6)	Linoleic acid	12.4
20:1 (.omega.9)	Eicosenoic acid	0.2
20:4 (.omega.6)	Arachidonic acid	4.2
22:6 (.omega.3)	DHA	5.8

EXAMPLE 2

EVALUATION OF MO-EM IN A DIC MODEL AND COMPARISON WITH INTRALIPID.RTM.

As a conclusion of previous experiments the most relevant way to follow the changes reflecting the DIC syndrome as induced by plasma kallikrein appear to be to perform consecutive determinations of the following hematological and hemostatical parameters: Fibrin monomer (FM, soluble fibrin); white blood cell count; fibrinogen; t-PA and .alpha.2-antiplasmin. Since platelets are most likely to be affected by the kallikrein (KK) injections, as indicated by the thromboxane metabolite excretion, it would probably be of interest to study to what extent this also affects the platelet function. Consecutive determinations of platelet aggregation ought to be included in a new study. In addition the cardiovascular parameters PAP, indicating the respiratory involvement of the DIC syndrome, BP, cardiac output (CO), heart rate, left ventricular pressure (LVP) and blood gases should be followed to reflect the involvement of the cardiovascular system.

EXPERIMENTAL PROCEDURE

Pigs, mean weight 26.3 kg, range 22-32 kg, n=19 were used for the experiments. The animals were given ketamin, 500 mg, (Ketalar, Parke-Davis, Morris Plains NJ) intramuscularly as a premedication. Anesthesia was induced with penthobarbital sodium, 5 mg/kg bw (Mebumal vet, ACO Stockholm Sweden) given intravenously and maintained with a continuous infusion of fentanyl, 10 mg/kg bw/h (Leptanal, Jansen Leo

Pharma AB, Helsingborg, Sweden) and pancuroniumbromide, 0.2 mg/kg bw/h (Pavulon, Organon, Oss, Netherlands). After induction of anesthesia the animals were all intubated and mechanically ventilated with an Engstrom respirator to an arterial carbon dioxide partial pressure of approximately 5 kPa with a gas mixture of O.sub.2 and N.sub.2 O 1:2. Catheters were placed in the mid aorta and inferior vena cava through a femoral cut down. A 7F triple-lumen catheter (Swan-Ganz, American Edwards Laboratories, Irve St Ana, Calif.) was introduced through a cut down to the right external jugular vein. Through a midline abdominal incision catheters were introduced into both ureters for control of diuresis and collection of urine. Arterial mean pressure (MAP), pulmonary artery mean pressure (PAP) and pulmonary capillary wedge pressure (PCWP) were recorded with capacitive transducers which were positioned at mid-thoracic level. All recordings were made with a Polygraph (Model 7B, Grass Instruments, Quincy, Mass). Arterial blood was drawn for blood gas analysis, made directly after sampling with a standard electrode technique (ABL 2, Radiometer, Copenhagen, Denmark) The animals were hydrated with isotonic saline to a stable wedge pressure. Cardiac output was measured by thermo dilution technique and a cardiac output computer was used for the calculations (model 9310 Edwards laboratories).

After the first blood sample was drawn and during the animal preparation a pretreatment period with infusions was started. During this period the animals received a high dose, 10 ml/kg bw, or a low dose, 5 mL/kg bw of lipid emulsion (see Example 1). The control group received 10 ml/kg bw of physiological saline solution and in order to give equivalent volumes to all animals the total dose of lipid emulsion and saline was adjusted to 10 ml/kg bw. These infusions were given over a 2-hour period after which there was a 1-hour stabilizing period before the kallikrein injection. Blood and urine were sampled before and 1 hour after infusions of lipid emulsions or saline (before kallikrein injection), and 30, 90 and 180 minutes after kallikrein injection.

Swine plasma kallikrein was isolated from pig plasma according to Gallimore et al (Thromb Res, 2, 409-420, 1978). It was dissolved in buffered saline to the concentration 0.9-1.1 units/ml (one unit is defined as the activity generated by total activation of the prekallikrein in 1 ml of pooled normal human plasma). Plasma kallikrein was diluted in 60 ml physiological saline and given as three 20 ml i v infusions over one minute at five minute intervals, in a total dose of 0.33 units/kg bw.

EXPERIMENTAL GROUPS AND DOSING

NaCl: Physiological saline, (control) 10 ml/kg bw, 0.08 ml/kg bw/min
OF-H: Marine oil emulsion, high dose 10 ml/kg bw, 0.08 ml/kg bw/min
IL-H: Intralipid .RTM. 20%, high dose 10 ml/kg bw, 0.08 ml/kg bw/min
OF-L: Marine oil emulsion, low dose, 5 ml/kg bw, 0.04 ml/kg bw/min
IL-L: Intralipid .RTM. 20%, low dose 5 ml/kg bw, 0.04 ml/kg bw/min

There were four animals in each group, except in the control group, there were three. The emulsions were prepared as described in Example 1.

The preparation of the emulsions was carried out in a conventional manner. The composition and preparation of marine oil emulsion is described in Example 1. Intralipid.RTM. contains 20% (w/v) oil as soybean oil and 1.2% (w/v) egg yolk phospholipids.

The infusion rate was four times higher than that recommended in normal clinical practice.

METHODS

White blood cell count as well as hematocrit determinations were performed in an electronic cell counter (Contrave Autolyzer 801, Zurich, Switzerland)

Fibrinogen was determined with a polymerization rate assay (Vermylen et al, Clin Chir Acta, 8, 418-424, 1973).

Soluble fibrin (fibrinmonomer) was determined by means of an amidolytic assay according to Wiman and R.ang.nby (Thromb Haemostas, 55, 189-193, 1986) utilizing kits from KabiPharmacia (Stockholm, Sweden, Coa-Set FibinMonomer)

Tissue plasminogen activator (t-PA) was determined by functional spectrophotometric methods utilizing kits from Biopool AB (Ume.ang. Sweden, Chmielewska et al, Clin Chem, 32, 482-485, 1986).

Alpha.sub.2 -Antiplasmin was determined by an amidolytic assay (Coatest Anti-plasmin, KabiPharmacia, Stockholm, Teger-Nilsson et al, J Clin Lab Invest, 47, 403, 1977).

Whole blood platelet aggregation was performed with ADP, 5 .mu.mol/l final concentration, in a Chrono-Log Whole Blood Agregometer (Coulter Electronics Ltd, Luton, UK, Cardinal et al, J Pharmacol Methods, 3, 135-137, 1980).

2,3-Dinor-thromboxane B.sub.2 and 2,3-dinor-prostaglandin F.sub.1 a, the major urinary metabolites of TxA.sub.2 and prostacyclin, were determined with quantitative gas chromatography and mass spectrometry (Vesterqvist and Green, Thromb Res, 33, 39-49, 1983; Prostaglandins 28, 139-154, 1984).

Blood samples. Arterial blood was drawn from an indwelling catheter. Nine parts of blood was mixed with one part trisodium citrate solution, 0.129 mol/l. Plasma was harvested after centrifugation and stored frozen at -70.degree. C. until analysis. Immediately after drawing and mixing with citrate solution, 1 ml of blood was taken for t-PA analysis and mixed with 0.5 ml sodium acetate buffer, 1 mol/l, pH 3.9. After centrifugation the supernatant was taken and stored at -70.degree. C.

Urine samples were obtained through catheters inserted into the ureters. A zero value was obtained by collecting the urine standing in the bladder.

Histopathology. Material fixed in 4% buffered neutral formaldehyde was received from the following organs: kidney, lung, heart and spleen (only one pig). The material was embedded in paraffin, sectioned in 4-5 .mu.m sections and stained with haematoxylin-eosin (HE), phosphotungstic acid haematoxylin (PTAH) and Martius scarlet blue (MSB). The two latter stains were used to demonstrate fibrin (Mallory, Pathological Technique, Saunders, 1938; Lendrum et al, J Clin, Path, 15, 401-413, 1962). The sections were examined under the light microscope. The treatments of the pigs were unknown to the examiner at the time of microscopical

examination.

RESULTS

Haematology

Effects on white blood cell count

The white blood cell count generally increased during the combined surgery, stabilizing and lipid infusion period as a response to the surgical trauma. After infusion of kallikrein the animals receiving control infusion or MO-em showed a progressive reduction of the white cell count reaching preinfusion level at 90 minutes post-(KK) infusion. At 180 min post-KK-infusion WBC count increased again in the placebo group, whereas it remained essentially stable in the other groups.

Platelet function tests

Platelet aggregation was reduced in all groups receiving lipid infusion except the low dose Intralipid.RTM. group, while it was essentially unchanged in the control group during the pre-kallikrein period, see FIG. 1. In the high dose MO-em group platelet aggregation was completely abolished after the lipid infusion. At 90 minutes after the kallikrein injection the platelet aggregation was lower in the control group than during the pre-kallikrein period but essentially unchanged for the lipid treated groups. The high dose MO-em and Intralipid.RTM. groups had regained some aggregability at 90 minutes post-kallikrein. The decrease in platelet aggregability at 90 minutes post-kallikrein may be explained by refractoryness and/or inhibition. In the control group, having high aggregability left before the kallikrein injection, many of the platelets may be refractorial to a new aggregation. However, in the MO-em groups, and possibly the high dose Intralipid.RTM. group, the aggregability was low before the kallikrein injection, indicating inhibition which remained also at 90 minutes post-kallikrein. The results show that even a short-lasting infusion of MO-em reduces platelet aggregation.

The main urinary metabolites of thromboxane (MUM-TXA) and prostacyclin (MUM-PGI) were slightly increased by kallikrein injection. This increase was not reduced by a short-lasting infusion of MO-em, at least not as measured in urine. The possibility remaines, however, for a local reduction.

Blood coagulation

The fibrinmonomer (FM), soluble fibrin, is a good marker for a generalized blood coagulation in vivo. FM was increased in all groups 30 minutes after kallikrein injection, indicating increased disseminated coagulation. The inability to show reduced blood coagulation 30 minutes after kallikrein injection and only 90 minutes after completed infusion of lipid emulsions may be explained by the remaining phospholipid vesicles in the blood (Osterud et al, 1990). The reduction in coagulability is however seen after oral (Osterud et al, 1990) administration of marine oil or 1 to 2 days after intravenously infused marine oil emulsion.

Fibrinolytic variables

Tissue plasminogen activator (t-PA) is released from vascular endothelial cells after various types of stimuli. t-PA activates plasminogen to plasmin with fibrinolytic activity. An increase in t-PA

was seen after kallikrein injection, with a maximum at 90 min. This increase was seen after infusion of MO-em in both doses but not after Intralipid.RTM.. During the pre-Kallikrein period after infusion of MO-em in high dose t-PA was increased to a level higher than the other groups, indicating stimulation of t-PA release, resulting in increased fibrinolysis. This was not seen after infusion of Intralipid.RTM.. At 180 minutes post-kallikrein the levels were normalised in all groups, see FIG. 2.

Antiplasmin which inactivates plasmin, was reduced 180 minutes after kallikrein injection, see FIG. 3. This reduction was similar in the MO-emulsion and the placebo groups. However, in the group which received a high dose MO-emulsion, antiplasmin was reduced more markedly, reflecting an increased plasmin generation and a more prominent fibrinolytic response. During the pre-kallikrein period, antiplasmin was reduced in all groups, indicating increased fibrinolysis, probably mainly due to surgery.

Fibrinogen which is converted to fibrin by the action of thrombin, was reduced in the control and MO-em groups during the pre-kallikrein period, presumably caused by the surgical trauma, see FIG. 4. The level remained low also after kallikrein injection. At 180 minutes post-kallikrein the level of fibrinogen was lower in the FO-H group than in the other group.

Histopathology

The number of pigs examined in each group was: two controls, four high dose MO-em, four high dose Intralipid.RTM., three low dose MO-em and three low dose Intralipid.RTM..

There was presence of fibrin-like material in small blood vessels in the heart in one of the two control pigs and in one of four pigs given the high dose and one of three pigs given the low dose, respectively, of Intralipid.RTM.. The deposition of fibrin-like material was slight in all three cases. Deposition of fibrin-like material was not observed in any pig given the MO-em.

To observe microthrombi formation in the circulation by light microscopy after only three hours of induction of DIC may be difficult and probably explain the relatively slight morphological manifestation of DIC in this study. MSB and PTAH stains are recommended in the diagnosis of DIC (Hamilton et al, J Clin Path, 31, 609-619, 1978; Skorten, Acta Path Microbiol Scand, 61, 405-414, 1964) although immunological methods are more specific. A few spontaneous changes were recorded in some pigs but were considered to be without importance for the evaluation of the effect of treatment of lipid emulsion in DIC.

In conclusion, no evident light microscopically visible morphological manifestations of fibrin depositions in the tissues examined from the MO-em treated pigs could be observed.

Cardiovascular parameters

1. Observations during the kallikrein infusion:

All animals had a fast rise in PAP, see FIG. 5, during the kallikrein infusion. The increase in PAP was less pronounced among the MO-em pretreated animals. All animals did also show a decrease in BP. The most

pronounced decrease in BP was seen in the high MO-em pretreated animals.

No major changes were observed in HR, WP, CO or PaO.sub.2 during this period.

2. Observations during the 180 minute post-Kallikrein injection period:

BP was restored to approximately the preinfusion values after about 30 minutes. PAP did also show a decline and the value was almost back to preinfusion level after about 180 minutes. Only minor changes were seen in wedge pressure, cardiac output and PaO.sub.2 during this period.

EXAMPLE 3

NEED OF ANTIOXIDANTS

Syndromes with DIC may include radical reactions. Oxygen and hydroxy radicals may induce lipid peroxidation of PUFA in cell membranes which as a consequence may lead to cell damage and induction of the cascade systems. Therefore it is important to protect the PUFA in the emulsion, during the administration and after incorporation into biological membranes. The present experiment describes the evaluation of the need of antioxidants to the marine oil emulsions.

Marine oil emulsions (MO-em) (see Example 1) were infused intravenously 20 hours/day to rats over 14 days. The MO-em:s differed in the type of antioxidant added. The daily dose was 25 ml (5 g TG)/kg body weight (b.w.) and the experimental groups were: A) MO-em without antioxidant; B) MO-em with a-tocopherol (vitamin E), 1 mg/g MO; C) MO-em with a-tocopherol, 1 mg/g MO, and vitamin C, 5 mg/g MO; D) MO-em with a-tocopherol, 5 mg/g MO; E) Intralipid.RTM. 20%; F) Physiological saline.

The results were the following;

1) Body weight, weight gain and organ weights (liver, spleen, kidney, lung, myocard, thymus) were similar in all groups.

2) The plasma level of vitamin E was lower in Group A but higher in Group D than in Groups E and F. The level of vitamin C in plasma did not change.

3) The level in the liver of malondialdehyde, MDA, a marker of lipid peroxidation, was higher in Group A compared to all other groups.

4) Histopathological changes consisted mainly of fatty changes in the liver. In Groups A-D these changes were more evident in the Kupffer cells than in the hepatocytes contrary to the findings in Group E. The granulomatous reaction in the liver was more pronounced in Group D than in the other groups.

EXAMPLE 4

DOSE-RESPONSE STUDY

Marine oil containing a high degree of **.omega.3-fatty acids** has anti-thrombotic and anti-**inflammatory** effects and effects on hemostasis and immune defence. In order to facilitate a more specific use of the **.omega.3-fatty acids** it is important to have information about the dose-response relationships for the various effects and possible side effects.

The aim of this study was to evaluate the dose-response relationship for

effects of the **.omega.3-fatty acids** on fatty acid incorporation, eicosanoid level (**inflammation**), hemostasis, immune defence and safety.

Dose-response relationships for biological effects induced by marine oil (MO)-emulsions, containing different amounts of marine oil triglycerides, (see Example 1) were evaluated. Three different 20% MO-emulsions, of which the composition of the oil was 100% (w/w) MO (Group A); 50% MO+50% soybean oil (SBO) (Group B); 10% MO+90% SBO (Group C) were infused intravenously to rats. The effects were compared with those induced by Intralipid.RTM. 20% (Group E) and physiological saline (Group F). The daily dose, 25 ml (5 g TG)/kg body weight (b w) in all groups was infused during 20 hours/day to rats during 14 consecutive days. All MO-emulsions contained .alpha.-tocopherol, 1 mg/g oil.

The results were as follows:

1) EPA and DHA in liver and spleen lipids increased dose-dependently in Groups A-C, whereas arachidonic and linoleic acids decreased, compared to Group F. Intralipid.RTM. (Group E) induced the opposite changes in these fatty acids. The fatty acid pattern was "normalized" by a low dose of MO-emulsion (Group C).

2) Red blood cell viscosity was reduced to a similar degree by the different MO-emulsions in Groups A-C.

3) The level of thromboxanes, which are prothrombotic in blood, was dose-dependently reduced by the different MO-emulsions with the threshold level in Group C.

4) MO-emulsion in the highest dose was more immunosuppressive than Intralipid.RTM.. Proliferation of splenic cells and thymocytes (3H-thymidin incorporation after Con A stimulation) was depressed more in group A, but similarly in Groups B and C, compared to Group E.

5) Final body weight, weight gain and the relative weights (g/kg b.w.) of liver, spleen and kidney were slightly higher in Groups A and B than in Groups E and F.

6) The degree and nature of fatty changes in Group C were similar to, whereas those in Groups A and B were somewhat more pronounced than those in Group E. The histopathological changes were considered to be slight and probably of no clinical relevance.

7) The amount of lipids in liver and spleen was similar in all groups.

8) The level of malondialdehyde, MDA, a marker of lipid peroxidation, was lower in Groups C and E and similar in Groups A and B to those in Group F.

9) The level in blood of vitamin E (.alpha.-tocopherol) was similar in Groups A-C to that in Groups E and F.

The results show that a relatively low dose of MO-emulsion can be chosen to increase the membranal content of **.omega.3-fatty acids** and reduce the RBC viscosity, leading to improved nutritive circulation, without risking the less desirable ones (immuno-suppression, histopathological changes, lipid peroxidation). A somewhat higher dose is needed for reduction of the level of thromboxanes.

SUMMARY

DIC is still frequently seen in combination with major trauma, septicemia, meningoencephalitis and pancreatitis. There is often however a 12-26 hours elapse between the onset of symptoms and the debut of coagulation and respiratory distress. Major efforts are today spent on therapeutic manoeuvres in order to prevent these disorders from appearing; early fracture stabilisation, antibiotics, pain management, stress reduction and the goal is also to optimize oxygen delivery. Prophylactic heparin therapy is also sometimes given. Still these syndromes appears in a non-systematic fashion and with a high frequency of most complicated course. Therefore it is most interesting, if some therapy could be given in advance to most patients at risk, with the potential effect of lowering the incidence of DIC.

We have now found that preparations containing **.omega.3-fatty acids** have advantageous effects in the treatment of DIC, which may be a symptom as such or included in other symptoms such as sepsis or ischemia. The effects of **.omega.3-fatty acids** are seen very early, already after two hours of infusion, and can thus be of value to reduce the incidence of, and as a treatment of the DIC syndrome which is mostly an acute situation. The effects of MO-emulsion in comparison to the control and Intralipid.RTM. are summarised in the table below.

TABLE 1

Positive effects of Mo-em in comparison to the control and to Intralipid .RTM.

Parameter	Effect of KK in the control group	At time (min) post - KK	Effects of Marine oil emulsion in comp. to placebo	in comp. to Intralipid .RTM.	Positive effects of MO
PAP	.uparw.	FO-H 5-30	FO-L .dwnarw.	FO-H .dwnarw.	FO-L .dwnarw. .dwnarw. Reduced vascular resistance
TPA	.uparw.	0-90	.uparw.	.uparw.	.uparw. Increased fibrinolysis
APL	.dwnarw.	180	.dwnarw.	.apprxeq.	.dwnarw. .dwnarw. Increased fibrinolysis
FBG	.dwnarw.	0-180	.dwnarw.	.apprxeq.	.dwnarw. .dwnarw. Reduced vulnerab. to PL.AG.

```
      --      0.sup.1  .dwnarw.
                              .dwnarw.
                                    .dwnarw.
                                          .dwnarw.
                                                Anti-
                                                thrombotic
                                                effect
FIBRIN
      .uparw. 180      .dwnarw.
                              .dwnarw.
                                    .dwnarw.
                                          .dwnarw.
                                                Reduced
DE-                                             DIC
POSITS                                          symptomes
```

.sup.1 post. emulsion inf. (0'); .dwnarw. lower; .uparw. higher; .apprxeq similar: -- no effect

An improvement of the DIC-syndrome was seen already after a two hours infusion of marine oil emulsion. The positive effects on hemostasis and DIC are obtained after a short single infusion, which together with the anti-**inflammatory** effects expected after repeated infusion may have wide implications on DIC related syndromes.

After two hours of infusion of marine oil emulsion the increase in PAP, induced by kallikrein-DIC, was reduced, indicating reduced ventilatory complications associated with DIC in the lungs. The reduction of the kallikrein induced increase in PAP and the reduction of MAP seen after infusion of MO-em indicates vasodilation induced by .**omega**.**3-fatty acids**. This effect may be explained either by reduced local production of TXA2 or induction of the release of NO. The results indicate that a short term infusion of a high dose of MO-em concomitant to an increase in plasma kallikrein and induction of DIC induces release of NO. The fibrinolytic response was increased and no fibrin deposits could be found in any of the organs tested (kidney, lung, heart and spleen). These end-point effects (reduced PAP, increased fibrinolysis, no fibrin deposits) show that the magnitude of the induced DIC symptoms was reduced. The tendency of the platelets to aggregate was minimized, indicating reduced risk of thrombosis. This effect may be explained by reduced TXA2 production in platelets or stimulated release of NO in endothelial cells. The reduced level of fibrinogen, seen after only two hours of infusion of marine oil emulsion, may together with other positive effects shown for .**omega**.**3-fatty acids** minimize the vulnerability for heart attacks. PaO.sub.2 was maintained. The reduction in RBC viscosity suggests increased nutritional blood flow through capillaries. The positive effects on hemostasis are obtained before, and with a lower dose than that needed to suppress the immune defence. Thus, by a short-term high dose of marine oil emulsion or long-term dosing of a mixture of marine oil and a vegetable oil in emulsion, the risk for immunosuppression is minimized. The effects seen after infusion of MO-em were dose-related and positive in comparison to the changes induced by kallikrein injection in the control and Intralipid.RTM. groups.

We have also shown that the addition of dl-.alpha.-tocopherol, 1 mg/g oil, is enough to counteract the peroxidation of the highly polyunsaturated fatty acids from marine oil and the reduction of endogenous .alpha.-tocopherol and vitamin C induced by MO-em. Furthermore, we have shown that the positive effects of .**omega**.**3-fatty acids** on DIC were obtained when

administered together with an antioxidant, .alpha.-tocopherol. From these results it may be concluded that the MO-em studied, containing dl-.alpha.-tocopherol, 1 mg/g oil, does not induce lipid peroxidation and is efficacious on DIC.

The positive effects of **.omega.3-fatty acids** on DIC are obtained after a single dose and before a reduction on the eicosanoid level is obvious. Thus, a low dose can be expected to be effective during long term treatment. If the dose is increased a reduction in eicosanoid synthesis and thereby the anti-**inflammatory** effect is obtained. This anti-**inflammatory** effect is in favour over that obtained with NSAID:s, since these drugs block only cyclooxygenase and thereby provide more substrate for the lipoxygenase pathway, leading to enhanced monocyte activation as expressed by increased lipoxygenase effects and induced tissue factor activity. A concomitant vasodilation and increase in nutritional blood flow, reduction in PAP, and maintained PaO.sub.2, is a great and unexpected advantage compared to present treatment with vasodilators, which usually reduce PaO.sub.2. Marine oil emulsions or other preparations containing **.omega.3-fatty acids** can thus be therapeutically useful to treat severe trauma and to help to prevent the development into various forms of DIC. Such emulsions can also be useful nutritionally for example to patients with DIC, or to patients vulnerable to the development of DIC, who also need TPN or in long term TPN, to reduce the symptoms of or to help to prevent the development into more chronic forms of DIC.

ABBREVIATION LIST

AA	Arachidonic acid
B.w.	Body weight
BP	Blood pressure
CO	Cardiac output
ConA	Concanavalin A
DHA	Docosahexaenoic acid
DIC	Disseminated intravascular coagulation
EDRF	Entothelial derived relaxing factor
EPA	Eicosapentaenoic acid
FM	Fibrin monomer
HE	Haematoxylin-eosin
IL-I	Interleukin I
IL	Intralipid .RTM.
IL-H	Intralipid, high dose
IL-L	Intralipid, low dose
KK	Kallikrein
LPS	Lipopolysacharides
LVP	Left ventricular pressure
MAP	Mean arterial pressure
MDA	Malondialdehyde
MO	Marine oil
MO-em	Marine oil emulsion
MO-H	Marine oil emulsion, high dose
MO-L	Marine oil emulsion, low dose
MOF	Multiorgan failure
MSB	Martius scarlet blue
MUM-PGI	Main urinary metabolit of prostacyclin
MUM-TXA	Main urinary metabolit of thromboxane
NO	Nitric oxide
NSAID	Nonsteroidal anti-**inflammatory** drugs
PaO.sub.2	Arterial pressure of oxygen
PAP	Pulmonary artery pressure
PCWP	Pulmonary capillary wedge pressure
PTAH	Phosphotungstic acid haematoxylin
PUFA	Polyunsaturated fatty acids

RBC	Red blood cells
t-PA	Tissue plasminogen activator
TF	Tissue factor
TG	Triglycerides
TNF	Tumor necrotic factor
TPN	Total parenteral nutrition
WP	Wedge pressure

CLM What is claimed is:

1. Method for treating or preventing the development of disseminated intravascular coagulation (DIC), which comprises administering to a patient in need thereof a preparation containing an effective amount of w 3-fatty acid, a salt or a derivative thereof.

2. Method according to claim 1, wherein the source of said . **omega.3-fatty acid** in said preparation is selected from group consisting of a marine oil, a vegetable oil, a synthetic triglyceride, a natural phospholipid and a synthetic phospholipid.

3. Method according to claim 1, wherein the preparation containing . **omega.3-fatty acids** is an emulsion, which is adapted for oral, peroral or parenteral administration containing conventional diluents, additives and emulsifiers.

4. Method according to claim 3, wherein the preparation containing . **omega.3-fatty acid** is adapted for total parenteral nutrition and optionally comprises .**omega.6-fatty acid.**

5. Method according to claim 2, wherein said preparation containing . **omega.3-fatty acid** is in the form of an aerosol for inhalation, a dosage form for nasal application or in a dosage form for oral or peroral administration.

6. Method according to claim 1, wherein said preparation containing . **omega.3-fatty acid** further contains an antioxidant.

7. Method according to claim 6, wherein said .**omega.3-fatty acid** is EPA or DHA or both, or derivatives or salts thereof.

8. Method according to claim 7, wherein said preparation is adapted for long-term treatment.

9. Method according to claim 5, wherein the dosage form for oral or peroral administration is tablet or capsule.

10. Method according to claim 2, wherein said preparation containing . **omega.3-fatty acid** further contains an antioxidant.

11. Method according to claim 10, wherein said .**omega.3-fatty acid** is EPA or DHA or both, or derivatives or salts thereof.

12. Method according to claim 11, wherein said preparation is adapted for long-term treatment.

13. Method according to claim 3, wherein said preparation containing . **omega.3-fatty acid** further contains an antioxidant.

14. Method according to claim 13, wherein said 3-fatty acid is EPA or DHA or both, or derivatives or salts thereof.

15. Method according to claim 14, wherein said preparation is adapted for long-term treatment.

16. Method according to claim 4, wherein said preparation containing .**omega.3-fatty acid** further contains an antioxidant.

17. The method of claim 3 wherein said emulsion comprises 0.5-50% (w/v) of an oil; 0.1-80% (w/v) of a phospholipid, at least 0.5% (w/v) of said .**omega.3-fatty acid** or salt or derivative thereof.

18. The method of claim 17 wherein said oil is present in an amount of 5-30% (w/v) and said phospholipid is present in an amount of 0.1-20% (w/v).

19. The method of claim 6 wherein said antioxidant is selected from the group consisting of .alpha.-tocopherol, vitamin C, a carotenoid and a retinoid.

20. The method of claim 3 wherein said emulsion is administered intravenously.

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INCLS: 514/458.000; 514/474.000; 514/560.000; 514/725.000
NCL NCLM: 514/549.000
NCLS: 514/458.000; 514/474.000; 514/560.000; 514/725.000
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ICM: A61K031-22
ICS: A61K031-355; A61K031-34; A61K031-20
EXF 514/549; 514/560; 514/474; 514/458; 514/725
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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AN 1998:31051 USPATFULL
TI Use of **omega-3-fatty acids**
IN Egberg, Nils, Lidingo, Sweden
Larsson-Backstrom, Carin, Stockholm, Sweden
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PA Pharmacia & Upjohn Aktiebolag, Stockholm, Sweden (non-U.S. corporation)
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RLI Division of Ser. No. US 1994-290905, filed on 21 Oct 1994
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DT Utility
FS Granted
REP

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EXNAM Primary Examiner: Weddington, Kevin E.
LREP Pollock, Vande Sande & Priddy
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 3 Drawing Page(s)

AB The present invention relates to **omega-3-fatty acid** containing preparations for the treatment of Disseminated Intravascular Coagulation (DIC) and symptoms related to DIC, as well as such preparations for reducing a pathological increase in pulmonary artery pressure (PAP). The preparations may be in the form of emulsions, or aerosols for inhalation, of an oil or phospholipids or other derivatives or salts of **omega-3-fatty acids** of marine and/or vegetable origin with a significant content of **omega-3-fatty acids**.
The preparations may also be in tablet or capsule form for oral use.

PARN This is a divisional application of Ser. No. 08/290,905, filed on Oct. 21, 1994.

SUMM The present invention relates to the use of **omega-3-fatty acids** (hereafter called **.omega.3-fatty acids**) for the treatment of or for preventing the development of Disseminated Intravascular Coagulation (hereinafter called DIC; for abbreviations, see the appended Abbreviation List) as well as reducing a pathological increase in pulmonary artery pressure (PAP). The preparations to be used may be in the form of emulsions for parenteral or enteral administration, or for example in the form of aerosols for inhalation or in a form for oral administration. The **.omega.3-fatty acids** (or salts or derivatives thereof) can originate from a marine or vegetable oil, from phospholipids, or be of syntheic origin.

BACKGROUND OF THE INVENTION

Today one of the major challenges for intensive care is to combat the secondary hypoperfusion syndromes seen after septicemia, trauma and malignancies. These syndromes include uncontrolled activation of the cascade systems (coagulation, fibrinolyses, kallikrein-kinin-, complement systems) often described as post traumatic micro embolism or disseminated intravascular coagulation (DIC).

Disseminated intravascular coagulation (DIC) gives rise to a wide variety of symptoms, to some extent caused by massive disseminated microembolism. There seem to be various target organs partly due to the inducing agent or cause as well as to probably a number of unknown factors.

A common situation in patients who develope DIC is pulmonary microembolism which could lead to severe problems of gas exchange, oedema and subsequent increase in pulmonary arterial pressure (PAP).

According the literature (Medicine, Edited by E Rubenstein and D D Federman published by Scientific American, New York, 1988, chapter 5:VI, Hemostasis and coagulation, p 35-38) there are a plurality of circumstances that can initiate the DIC-syndrome. Such circumstances may be massive tissue damage, leading to the release of huge amounts of tissue thromboplastic materials, causing extensive activation of the extrinsic system or extensive destruction of endothelial surfaces. The circumstances can be caused by for example severe injuries and infections, tumor products, hemolytic transfusion reactions, vasculitis, heatstroke, hemangomias and certain snake bites. In all cases this leads to a massive activation of the hemostatic mechanisms, which overwhelms the inhibitor mechanisms.

The entire scheme of coagulation either initiated by the intrinsic, including the kallikrein-Factor XII, pathway or extrinsic pathways is finely tuned to culminate in a burst of thrombin activity, causing hemostatic activity at the site of the injury, which leads to deposition of cross-linked fibrin to form a hemostatic plug. Normally, the effects of intravascular coagulation are controlled or modulated by the dilutional effects of the blood flow, by antithrombines, antiplasmin and among other factors the mechanisms that down-regulate hemostasis. However, these control mechanisms can be overwhelmed and disordered by the circumstances mentioned above. This may lead to excessive release of thrombin, which results in thromboses, ischemic conditions and necrosis.

The DIC syndrome can thus lead to massive intravascular deposition of

fibrin and impaired nutritive circulation leading to organ failure. This picture of DIC has for a long time dominated the concept of this common disorder. However, alternative views on this syndrome has also been brought forward. The fact that probably several other enzyme systems are involved in the syndrome has been focused on by suggestions on alternate names like "defibrination syndrome". This is discussed in the article of G. Muller-Berghaus in Seminars In Thrombosis and Hemostasis, vol. 15, No. 1, 1989, page 58-87, which is referred to for a review of the numerous conditions related to DIC.

Despite modern treatment modalities, the high mortality rate (>50%) from DIC has not decreased appreciably over the last 20 years. A great number of various therapies have been tried in order to prevent and also treat this syndrome. The treatment of DIC has for a long time been focused on an inhibition of the coagulation process by means of administration of heparin, antithrombin concentrates or hirudin. Depending on the activation mechanism(s) and the dominating symptoms alternative treatments have been suggested like dextran, acetylsalicylic acid, aprotinin, tranexamic acid and even streptokinase. Today corticoid steroid treatment is one of the pharmacological interventions that are frequently tried on these syndromes (C Putterman, J Critical Care 5 (4), 241-251, 1990). Until now however, there has been no consensus how to combat these syndromes. Furthermore there are no scientific proofs for any specific regim in order to prevent this syndrome. Aggressive fracture stabilisation, optimal pain relief and adequate antibiotic therapy is of course of vital importance.

A most interesting report by C Esmon and co-workers (Thrombos Haemostas 66 (1), 160-165, 1991) demonstrated the effect of Protein C.sub.a on an experimental animal model where a disseminated intravascular coagulation was induced by infusion of endotoxin from E. coli bacteria. It was shown, that unlike heparin, which only prevented the fibrin formation but not the shock, Protein C.sub.a prevented the development of the whole syndrome. It was further suggested that the dramatic effect of Protein C.sub.a was probably due to a combined inhibitory effect on hemostasis and on the **inflammatory** reactions included in the endotoxin induced syndrome.

Another approach for the treatment of DIC has essentially aimed at a substitution of consumed coagulation factors and inhibitors. Substitution has been given as full plasma or plasma concentrates of coagulation factors or antithrombin. An adequate treatment of the underlying disease or trauma as well as good general care for circulation and ventilation, has on the other hand been shown to be the most effective way to eradicate this dangerous complication.

Different fatty acids in the lipids have different physiological, biochemical and pharmacological properties and during the last years great interest has been concentrated on the importance of the polyunsaturated **.omega.3-fatty acids**, containing 18-22 carbon atoms. The **.omega.3-fatty acids** eicosapentaenoic acid (20:5 .omega.3, EPA) and docosahexaenoic acid (22:6 .omega.3, DHA) are essential fatty acids in man. Besides their nutritional value, they are also known to possess pharmacological effects. The best known are the cardiovascular effects, the beneficial effects on **inflammatory** and autoimmune diseases and the necessity of these fatty acids for the normal development of brain and retina functions. These effects have

such an importance that a lot of work has been done to find good nutritional compositions containing a high amount of **.omega.3-fatty acids**. See e g WO 87/02247 (Baxter) and U.S. Pat. No. 4,820,731 (New England Deaconess Hospital) in which marine oils are used which contain a high amount of the **.omega.3-fatty acids** EPA and DHA.

Early observations by Dyerberg et el. (Lancet, ii;117-119, 1978) indicated that there was an association between a high intake of **.omega.3-fatty acids** and prolonged bleeding time in Greenland Eskimos. One explanation for the prolonged bleeding time was further shown to be a suppression of the thromboxane A.sub.2 (TxA.sub.2) synthesis leading to an impaired platelet function. When fish oils are ingested and EPA displaces arachidonic acid (AA), precursor for eicosanoides, from cell membrane phospholipids, eicosanoides from a different series, 3-series, are produced. The thromboxane formed from EPA, unlike that from AA, has very little physiologic activity, whereas the prostacyclin is fully active, leading to an increased total antithrombotic and antiatherogenic prostacyclin activity (Leaf & Weber, n-3 News vol III (4), 1988). However, the **.omega.3-fatty acids** in fish oil may influence blood dotting, thromboses and fibrinolysis in many ways.

Tissue plasminogen activator (t-PA) is released from vascular endothelial cells after various kinds of stimuli. Plasminogen is activated by t-PA to plasmin which is the fibrinolytically active enzyme. The thrombolytically active t-PA can cause a dissolution of dots within blood vessels and, thus, prove useful in acute treatment of heart attacs. Dietary supplements for weeks of fish oil has been shown to increase endogenous t-PA production (Barcelli et al, Thromb Res, 39, 307-312, 1985). This action should be the major deterrent to the development of blood clots in coronary arteries which are usually the terminal events blocking blood flow to the heart muscle thus causing heart attacks.

An elevated level of plasma fibrinogen has been identified as a risk factor for coronary artery disease. Dietary intake of fish oil for weeks suppresses the fibrinogen level (Hostmark et el, Br Med I (Clin Res) 297 (6642), 180-181, 1988). Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are both involved in the development of an **inflammatory** response. Dietary intake of fish oil for weeks has been shown to reduce the production of IL-1 and TNF (Endres et el, Clin Immunol Immunopath, 49, 424-438, 1989).

The endothelial derived relaxing factor (EDRF), recently shown to be identical to nitrogen oxide (NO), has a relaxing effect on vascular smooth muscle and counteracts agents causing vasoconstriction resulting in hypoxic vascular damage. NO has also an antithrombotic and cytotoxic action. The cytotoxicity of activated macrophages against tumor target cells was shown to be dependent on the presence of NO (for references see Moncada et al., Pharmacological Reviews, vol.43, No 2, 1991). Fish oil feeding for weeks is claimed to enhance the effect of EDRF (Vanhoutte et al, In: Health effects of .omega.3 polyunsaturated fatty acids in seafoods. Eds Simopoulos et al, Karger, 233-244, 1991). Tissue factor (TF), earlier referred to as tissue thromboplastin, is a potent trigger of the extrinsic pathway of blood coagulation. TF is produced by a large number of cell types, though not endothelium. Monocytes can be stimulated by lipopolysaccharides (LPS), which are toxic bacterial material, to expression of TF. Liposomes prepared from soybean lecithin can enhance the LPS effect of inducing thromboplastin in monocytes in the blood. TF expression, as induced by LPS and liposomes, was reduced

by 40% after 8 weeks of fish oil diet (Osterud et al, Omega-3 News vol V (2),1990). The mechanism for this inhibition probably includes an inhibition of arachidonic acid metabolism to eicosanoides, by cyclo-oxygenase to prostanoides and by lipoxygenase to leucotrienes (Osterud et al, Abb Med, 21, 47, 1989). Non-steroidal anti-**inflammatory** drugs (NSAID), like acetylsalicylic acid, however, block only the cyclo-oxygenase and thereby provide more substrate for the lipoxygenase pathway, leading to 50-250% enhanced monocyte activation as expressed by induced thromboplastin activity. Thus, to create an inhibition on **inflammatory** reactions as well as on blood coagulation, fish oil might be a better treatment than NSAID.

The above mentioned mechanisms are involved in reactions which may lead to the DIC syndrome.

In an animal experimental model we have previously studied a DIC-like syndrome induced by infusion of plasma kallikrein (Egberg et al, Fibrinolysis, 2, 95-100; 101-106, 1988). In the initial studies we followed the plasma concentrations of prekallikrein and free kallikrein activity as well as coagulation factor XII and fibrinogen. The major inhibitor of plasma kallikrein, C.sub.1 -esterase inhibitor, as well as antithrombin, .alpha..sub.1 -antitrypsin and .alpha..sub.2 -macroglobulin were also determined. Fibrinolytic variables like plasminogen and .alpha..sub.2 -antiplasmin were additionally followed. The conclusions drawn from these studies were that a slowly professing DIC was induced leading to a small but progressive consumption of coagulation factors. There was also a progressive consumption of .alpha..sub.2 -antiplasmin, indicating a comparatively intense activation of the fibrinolytic system. These findings lead to the conclusions that we should look closer for a possible platelet activation. This was done by following the urinary excretion of the major thromboxane A.sub.2 metabolite, 2,3-dinor-tromboxane B.sub.2. Thromboxane A.sub.2 is synthetized by platelets after various types of platelet stimulation and is one of the most powerful platelet aggregating agents known. At the same time we followed the urinary excretion of the major metabolite of prostacyclin, 2,3-dinor-prostaglandin F.sub.1 a. Prostacyclin is synthetized and released from the vascular endothelium and is possibly the most effective inhibitor of platelet aggregation in the body. We also decided to look at the activation of the fibrinolytic system by measuring the plasma level of tissue plasminogen activator, t-PA.

In the previous study on a DIC-like syndrome we found a rise of the urinary excretion of thromboxane and prostacyclin metabolites after the kallikrein injections, indicating that the arachidonic acid metabolism was stimulated. The increased excretion rate for thromboxane metabolites suggested an in vivo aggregation of platelets that could contribute to the syndrome developed. A marked fall of the leucocyte count, which may be explained by aggregation of leucocytes, was also found.We also found an increase of the plasma level of t-PA, which probably was the cause of the earlier observed signs of activation of the fibrinolytic system. Of the cardiovascular parameters measured pulmonary artery pressure (PAP) increased and blood pressure (BP) was reduced.

DESCRIPTION OF THE INVENTION

We have found surprisingly that preparations containing **.omega .3-fatty acids** have extraordinarly advantageous effects for the treatment of DIC including a reduced pathological increase in pulmonary artery pressure (PAP).

In the present investigation we have Studied the changes in the

hemostatic system in a DIC model induced by injection of plasma kallikrein after pretreating the animals with marine oil emulsion.

We claim the use of **.omega.3-fatty acids** for the preparation of a medicament to be useful in the treatment of, or for preventing the development of DIC or for reducing the pathologically increased PAP. The **.omega.3-fatty acids** may come from marine oils, vegetable oils rich in **.omega.3-fatty acids** or from phospholipids containing **.omega.3-fatty acids** . The **.omega.3-fatty-acids** may also be in the form of synthetic derivatives or salts thereof.

Suitable administration forms are emulsions for parenteral, peroral or oral use, where the emulsions may be of therapeutic value or adapted for TPN (Total Parenteral Nutrition). Other suitable administration forms are inhalable aerosols, dosage forms to be administered nasally and orally in the form of tablets and capsules.The preferred **.omega.3-fatty acids** are EPA and/or DHA or their salts or derivatives.

The beneficial effects of fish oil after oral administration are first obtained after weeks of treatment. In order to reduce the incidence of DIC, which is mostly an acute situation, the effects of fish oil should be obtained with a short onset of action. The **.omega.3-fatty acids** ought therefore to be active in some intravenously or inhalalable active and tolerable administrative form.

In the present invention we have used **.omega.3-fatty acids** in a preparation derived from marine oils in the form of an oil in water emulsion as prepared and presented below in Example 1. It must be clearly stated that other preparations containing **.omega.3-fatty acids** or derivatives thereof must be considered to be useful in the treatment of DIC. Such preparations may be types of emulsions or solutions other than specified in Example 1, with more concentrated **.omega.3-fatty acids** , with appropriate diluents or carriers, as well as oral preparations containing the **.omega.3-fatty acids** as salts of glycerol- and ethyl esters, phospholipids or sterols or other derivatives of **.omega.3-fatty acids** and suitable exdpients. The oral preparations may be in conventional tablet form or in capsules manufactured according to well known techniques.

As alternatives to the mentioned preparations aerosols can be effective, both in a conventional form and in a form where the **.omega.3-fatty acids** are comprised in eventually bilayer forming phospholipids (liposomes) and different nasal preparations. The aerosols are intended to be adminstered by inhalation to the lungs, but may also be adapted to be administered through the nasal mucous membranes.

Useful emulsions could comprise 0.5-50% (w/v of total emulsion) oil, preferably 5-30% (w/v), vegetable oils, such as soybean oil, coconut oil, cottonseed oil, safflower oil, sunflower seed oil, linseed oil, borage oil, blackcurrent seed oil, canola oil or other vegetable oils containing **.omega.3-fatty acids**, or marine oil, or a mixture of those mentioned. The amount of the phospholipids could be 0.1-80% (w/v of total emulsion), preferably 0.1-20% (w/v). The preparation should contain **.omega.3-fatty acids** or derivatives thereof to an amount of 0.5-100%.

Phospholipids such as egg yolk or soybean phospholipids, marine phospholipids or synthetic emulsifiers can also be included in the emulsion. The total amount of emulsifier is preferably 0.1-20% (w/v of total emulsion). The emulsion can also contain other components which are normally incorporated in emulsions e.g. monoglycerides of fatty acids, components for adjusting isotonic properties (such as glycerol), antioxidants such as .alpha.-tocopherol, components for adjusting stability such as amino acids, and carbohydrates such as fructose and glucose etc.

Antioxidants should be added to protect the unsaturaed **.omega.3-fatty acids** from oxidation. Such antioxidants could be .alpha.-tocopherol (Vitamin E), Vitamin C, carotenoides or retinoides. However, other antioxidants can be used which are active to protect the unsaturated **.omega.3-fatty acids** from oxidation in the preparation, after administration and after incorporation into biological membranes. A study of antioxidants in marine oil emulsions has been performed and is presented in Example 2 below.

The preparation of the emulsion is carried out in a conventional manner.

Thus the lipids are mixed with the aqueous phase, phospholipids and optionally other emulsifiers and auxiliary agents in a suitable mixing device. Then the blend is homogenized to a desired particle size, preferably less than 1 micron. The ways to adjust the emulsion to a suitable particle size is well known to a person skilled in the art.

DIC is frequently still seen in combination with major trauma, septicemia, meningoencephalitis and pancreatitis. There is often, however, a 12-26 hours elapse between the onset of symptoms and the debut of coagulation and respiratory distress. Major efforts are today spent on therapeutic manoeuvres in order to prevent these disorders from appearing, by early fracture stabilisation, antibiotics, pain management, stress reduction and the goal is also to optimize oxygen delivery. Still these syndromes appear in a non-systematic fashion and with a high frequency of most complicated course. Therefore it seems most interesting, that some therapy could be given in advance to most patients at risk, with the potential effect of lowering the incidence of DIC.

We have now found that preparations containing **.omega.3-fatty acids** have advantageous effects for the prevention of or the treatment of DIC, so that the incidence of DIC will be reduced. The DIC syndrome may be a syndrome as such or included in other syndromes like pulmonary microembolization, multiple organ failure (MOF), sepsis and other infectious and ischemic conditions. The effects of **.omega.3-fatty acids** are seen very early, already after two hours of infusion, and can thus be of value for treatment and prevention of the development of the DIC syndrome and to reduce the incidence of DIC and related syndromes, which mostly are acute situations.

The preparations containing **.omega.3-fatty acids** will also be useful in the treament of DIC-related symptoms and condtions, such as incresed pulmory arterial pressure (PAP) and those described in the articles in Seminars In Thrombosis And Hemostasis vol. 14, No. 4, 1988, pages 299-338 (R L Bick) and in Seminars In Thrombosis And Hemostasis vol. 15, No. 1, 1989, pages 58-87 (G Muller-Berghaus).

The **.omega.3-fatty acids** in fish

oil may influence on blood dotting, thrombosis and fibrinolysis in many ways. The beneficial effects of fish oil is relevant for the treatment of the DIC syndrome. The **.omega.3-fatty acids** may reduce the hemostatic changes so that coagulation, fibrinolysis and thrombosis parameters are kept in balance. The end points of these effects are reduction of fibrin deposits, reduced microembolism and reduced tendency for increased pulmonary artery pressure and to prevent a decrease in PaO.sub.2. In subchronic/chronic cases of DIC (see Seminars In Thrombosis And Hemostasis, vol. 14, No. 4, 1988, R. L. Bick), of which some patients are treated with total parenteral nutrition (TPN), e.g. malignancies (gastrointestinal, pancreas, prostate, lung, breast), chronic **inflammatory** disorders (Chrons disease), the effects of **.omega.3-fatty acids** may be obtained in lower doses administered for a longer period of time. In more acute situations with DIC (see the mentioned article of R. L. Bick, 1988), e.g. bacteremia, bums, disseminated malignancy, liver disease, vascular disorders, the effects of fish oil should be elaborated rapidly enough to be of value to reduce the incidence of DIC.

In order to obtain an acute effect the **.omega.3-fatty acids** should be administered intravenously in the form of an emulsion. The acute effect should appear within hours and therefore the dose needed can be expected to be relatively high. In the experiments performed and presented below in Example 4 it is shown an improvement of the DIC syndrome already after a two hour infusion of marine oil emulsion. The PAP and thus the ventilatory complications associated with DIC in the lungs were reduced, PaO.sub.2 was maintained, the tendency of the platelets to aggregate was minimized, the fibrinolytic response was increased and no fibrin deposits could be found in any of the organs tested (kidney, lung, heart and spleen). The reduced level of fibrinogen, seen after only two hours of infusion of marine oil emulsion, may together with other positive effects shown for **.omega.3-fatty acids**, minimize the vulnerability for heart attacks. The reduction in RBC viscosity indicates increased nutritional blood flow through the capillaries. The positive effects on hemostasis are obtained before, and with a lower dose than that needed to suppress the immune defence.

The positive effects on hemostasis and DIC are obtained after a short single infusion, which together with the anti-**inflammatory** effects expected after repeated infusions may have wide implications on DIC related symptoms. This anti-**inflammatory** effect is in favour over that obtained with NSAID:s, since these drugs block only cyclo-oxygenase and thereby provides more substrate for the lipoxygenase pathway, leading to enhanced monocyte activation as expressed by increased lipoxygenase effects and induced tissue factor activity. A concomitant vasodilation and increase in nutritional blood flow, reduction in PAP and maintained PaO.sub.2 is a great and totally unexpected advantage compared to present treatment with vasodilators, which usually reduce PaO.sub.2. This, together with increased fibrinolysis, reduced thrombogenicity and fibrin deposits, and the rapid onset of action enables the long-chain polyunsaturated fatty acids a unique and unexpected possibility to treat and prevent the development of DIC.

Lipid emulsions or other preparations containing **.omega.3-fatty acids**, such as aerosols for inhalation, containing **.omega.3-fatty acids**, are useful therapeutically to treat severe trauma and to treate and help to prevent the development of various forms of DIC. Such

emulsions are also nutritionally useful, for example to patients with DIC, who also need parenteral nutrition (TPN) for a shorter period, or in long term TPN to reduce the symptoms of more chronic forms of DIC.

The invention thus relates to the use of **.omega.3-fatty acids** or their derivatives in emulsions or in other preparations with therapeutic effects for various forms of DIC and DIC-related symptoms as incresed PAP or to reduce the incidence of these symptoms and also, in combination with TPN, to these patients. The administration form can be by parenteral infusion or inhalation of aerosols containing **.omega.3-fatty acid** rich phospholipids or nasal preparations to thereby obtaining acute as well as chronic, long-lasting effects, or by peroral or oral administration in more chronic situations with DIC or in inhalations of liposomes as **.omega.3-fatty acid** containing phospholipids to reduce the risk of complications related to pulmonary microembolization. The doses of **.omega.3-fatty acids** to be administered in an acute situation (1-2 days) may be high in order to approach. The level of the therapeutic window. For therapeutic use over a longer time period with repeated administration the dose of **.omega.3-fatty acids** may be reduced to approach the amount of **.omega.3-fatty acids** which should be of not only therapeutic but also of nutritional value. For nutritional use in TIN the **.omega.3-fatty acids** should be administered together with other fatty acids.

Various modifications and equivalents of the emulsion or other forms of therapeutic preparations will be apparent to one skilled in the art without departing from the spirit or scope of the invention. It is therefore to be understood that the invention is not to be limited to the specific examples and embodiments disclosed herein.

DETD EXAMPLES

Example 1

Preparation of an emulsion containing fish oil and egg yolk phospholipids

The emulsion contained:

Fish oil	200	g
Egg yolk phospholipids	12.0	g
Glycerol	22.2	g
Aq. ad inject.	750	g
NaOH, 1 M	1.3	ml

As antioxidant vitamin E (.alpha.-tocopherol) was added to the emulsion in an amount stated in me respective example.

The ingredients above were mixed in a "Ultra Turrax" and thereafter homogenized in a "Moulin-Gaulin High Pressure Homogenizer" The fish oil used had the following fatty acid content in %:

14:0	Myristic acid 6.3
16:0	Palmitic acid 14.7
16:1 (.omega.7)	Palmitoleic acid

		7.3
18:0	Stearic acid	2.6
18:1 (.omega.9)	Oleic acid	8.9
18:1 (.omega.7)	Vaccenic acid	3.1
18:2 (.omega.6)	Linoleic acid	1.1
18:3 (.omega.3)	**Linolenic acid**	0.7
18:4 (.omega.3)	Stearidonic acid	2.6
20:1 (.omega.9)	Eicosenoic acid	1.5
20:4 (.omega.6)	Arachidonic acid	1.4
20:5 (.omega.3)	EPA	17.8
22:1 (.omega.11)	Docosaenoic acid	2.2
22:5 (.omega.3)	Docosapentaenoic acid	2.9
22:6 (.omega.3)	DHA	13.5

Total amount of fatty acids: 100% (w/w).

The egg yolk phospholipids used had the following fatty acid content in % of total fatty acids (w/w):

14:0	Myristic acid	0.2
16:0	Palmitic acid	31.5
16:1 (.omega.7)	Palmitoleic acid	1.2
18:0	Stearic acid	14.1
18:1 (.omega.9)	Oleic acid	28.0
18:2 (.omega.6)	Linoleic acid	12.4
20:1 (.omega.9)	Eicosenoic acid	0.2
20:4 (.omega.6)	Arachidonic acid	4.2
22:6 (.omega.3)	DHA	5.8

Example 2

Evaluation of MO-em in a DIC model and comparison with Intralipid.RTM.

As a conclusion of previous experiments the most relevant way to follow the changes reflecting the DIC syndrome as induced by plasma kallikrein appear to be to perform consecutive determinations of the following hematological and hemostatical parameters: Fibrin monomer (FM, soluble fibrin); white blood cell count; fibrinogen; t-PA and .alpha.2-antiplasmin. Since platelets are most likely to be affected by the kallikrein (KK) injections, as indicated by the thromboxane metabolite excretion, it would probably be of interest to study to what extent this also affects the platelet function. Consecutive determinations of platelet aggregation ought to be included in a new study. In addition the cardiovascular parameters PAP, indicating the respiratory involvement of the DIC syndrome, BP, cardiac output (CO), heart rate, left ventricular pressure (LVP) and blood gases should be followed to reflect the involvment of the cardiovascular system.

Experimental Procedure

Pigs, mean weight 26.3 kg, range 22-32 kg, n=19 were used for the experiments. The animals were given ketamin, 500 mg, (Ketalar, Parke-Davis, Morris Plains N.J.) intramuscularly as a premedication. Anesthesia was induced with penthobarbital sodium, 5 mg/kg bw (Mebumal vet, ACO Stockholm Sweden) given intravenously and maintained with a continous infusion of fentanyl, 10 mg/kg bw/h (Leptanal, Jansen Leo Pharma AB, Helsingborg, Sweden) and pancuroniumbromide, 0.2 mg/kg bw/h (Pavulon, Organon, Oss, Netherlands). After induction of anesthesia the animals were all intubated and mechanically ventilated with an Engstrom respirator to an arterial carbon dioxide partial pressure of approximately 5 kPa with a gas mixture of O.sub.2 and N.sub.2 O 1:2. Catheters were placed in the mid aorta and inferior vena cava through a femoral cut down. A 7F triple-lumen catheter (Swan-Ganz, American Edwards Laboratories, Irve St Ana, Calif.) was introduced through a cut down to the right external jugular vein. Through a midline abdominal incision catheters were introduced into both ureters for control of diuresis and collection of urine. Arterial mean pressure (MAP), pulmonary artery mean pressure (PAP) and pulmonary capillary wedge pressure (PCWP) were recorded with capacitive transducers which were positioned at mid-thoracic level. All recordings were made with a Polygraph (Model 7B, Grass Instruments, Quincy, Mass.). Arterial blood was drawn for blood gas analysis, made directly after sapling with a standard electrode technique (ABL 2, Radiometer, Copenhagen, Denmark) The animals were hydrated with isotonic saline to a stable wedge pressure. Cardiac output was measured by thermo dilution technique and a cardiac output computer was used for the calculations (model 9310 Edwards laboratories).

After the first blood sample was drawn and during the animal preparation a pretreatment period with infusions was started. During this period the animals received a high dose, 10 ml/kg bw, or a low dose, 5 mL/kg bw of lipid emulsion (see Example 1). The control group recieved 10 ml/kg bw of physiological saline solution and in order to give equivalent volumes to all animals the total dose of lipid emulsion and saline was adjusted to 10 ml/kg bw. These infusions were given over a 2-hour period after which there was a 1-hour stabilizing period before the kallikrein injection. Blood and urine were sampled before and 1 hour after infusions of lipid emulsions or saline (before kallikrein injection), and 30, 90 and 180 minutes after kallikrein injection.

Swine plasma kallikrein was isolated from pig plasma according to Gallimore et al (Thromb Res, 2, 409-420, 1978). It was dissolved in buffered saline to the concentration 0.9-1.1 units/ml (one unit is defined as the activity generated by total activation of the prekallikrein in 1 ml of pooled normal human plasma). Plasma kallikrein

was diluted in 60 ml physiological saline and given as three 20 ml i v infusions over one minute at five minute intervals, in a total dose of 0.33 units/kg bw.

Experimental groups and dosing

NaCl:	Physiological saline, (control) 10 ml/kg bw, 0.08 ml/kg bw/min
FO-H:	Marine oil emulsion, high dose
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IL-H:	Intralipid .RTM. 20%, high dose
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FO-L:	Marine oil emulsion, low dose, 5 ml/kg bw, 0.04 ml/kg bw/min
IL-L:	Intralipid .RTM. 20%, low dose
"-	

There were four animals in each group, except in the control group, there were three. The emulsions were prepared as described in Example 1.

The preparation of the emulsions was carried out in a conventional manner. The composition and preparation of marine oil emulsion is described in Example 1. Intralipid.RTM. contains 20% (w/v) oil as soybean oil and 1.2% (w/v) egg yolk phospholipids.

The infusion rate was four times higher than that recommended in normal clinical practice.

Methods

White blood call count as well as hematocrit determinations were performed in an electronic cell counter (Contrave Autolyzer 801, Zurich, Switzerland)

Fibrinogen was determined with a polymerization rate assay (Vermylen et al, Clin Chir Acta, 8, 418-24, 1973).

Soluble fibrin (fibrinmonomer) was determined by means of an amidolytic assay according to Wiman and Ranby (Thromb Haemostas, 55, 189-193, 1986) utilizing kits from KabiPharmacia.(Stockholm, Sweden, Coa-Set FibinMonomer)

Tissue plasminogen activator (t-PA) was determined by functional spectrophotometric methods utilizing kits from Biopool AB (Umea Sweden, Chmielewska et al, Clin Chem, 32, 482-485, 1986).

Alpha.sub.2 -Antiplasmin was determined by an amidolytic assay (Contest Antiplasmin, KabiPharmacia, Stockholm, Teger-Nilsson et al, J Clin Lab Invest. 47, 403, 1977).

Whole blood platelet aggregation was performed with ADP, 5 .mu.mol/l final concentration, in a Chrono-Log Whole Blood Agregometer (Coulter Electronics Ltd, Luton, UK, Cardinal et al, J Pharmacol Methods, 3, 135-137, 1980).

2,3-Dinor-thromboxane B.sub.2 and 2,3-dinor-prostaglandin F.sub.1 a, the major urinary metabolites of TxA.sub.2 and prostacyclin, were determined with quantitative gas chromatography and mass spectrometry Vesterqvist and Green, Thromb Res, 33, 39-49, 1983; Prostaglandins 28, 139-154,

1984).

Blood samples. Arterial blood was drawn from an indwelling catheter. Nine parts of blood was mixed with one part trisodium citrate solution, 0.129 mol/l. Plasma was harvested after centrifugation and stored frozen at -70.degree. C. until analysis. Immediately after drawing and mixing with citrate solution, 1 ml of blood was taken for t-PA analysis and mixed with 0.5 ml sodium acetate buffer, 1 mol/l, pH 3.9. After centrifugation the supernatant was taken and stored at -70.degree. C.

Urine samples were obtained through catheters inserted into the ureters. A zero value was obtained by collecting the urine standing in the bladder.

Histopathology. Material fixed in 4% buffered neutral formaldehyde was received from the following organs: kidney, lung, heart and spleen (only one pig). The material was embedded in paraffin, sectioned in 4-5 .mu.m sections and stained with haematoxylin-eosin (HE), phosphotungstic acid haematoxylin (PTAH) and Martius scarlet blue (MSB). The two latter stains were used to demonstrate fibrin (Mallory, Pathological Technique, Saunders, 1938; Lendrum et al, J Clin, Path, 15, 401-413, 1962). The sections were examined under the light microscope. The treatments of the pigs were unknown to the examiner at the time of microscopical examination.

Results

Haematology

Effects on white blood cell count

The white blood cell count generally increased during the combined surgery, stabilizing and lipid infusion period as a response to the surgical trauma. After infusion of kallikrein the animals receiving control infusion or MO-em showed a progressive reduction of the white cell count reaching preinfusion level at 90 minutes post-(KK) infusion. At 180 min post-KK-infusion WBC count increased again in the placebo group, whereas it remained essentially stable in the other groups.

Platelet function tests Platelet aggregation was reduced in all groups receiving lipid infusion except the low dose Intralipid.RTM. group, while it was essentially unchanged in the control group during the pre-kallikrein period, see FIG. 1. In the high dose MO-em group platelet aggregation was completely abolished after the lipid infusion. At 90 minutes after the kallikrein injection, the platelet aggregation was lower in the control group than during the prekallikrein period but essentially unchanged for the lipid treated groups. The high dose MO-em and Intralipid.RTM. groups had regained some aggregately at 90 minutes post-kallikrein. The decrease in platelet aggregability at 90 minutes post-kallikrein may be explained by refractoryness and/or inhibition. In the control group, having high aggregability left before the kallikrein injection, many of the platelets may be refractorial to a new aggregation. However, in the MO-em groups, and possibly me high dose Intralipid.RTM. group the aggregability was low before the kallikrein injection, indicating inhibition which remained also at 90 minutes post-kallikrein. The results show that even a short-lasting infusion of MO-em reduces platelet aggregation.

The main urinary metabolites of thromboxane (MIM-TXA) and prostacyclin

(MUM-PGI) were slightly increased by kallikrein injection. This increase was not reduced by a short-lasting infusion of MO-em, at least not as measured in urine. The possibility remaines, however, for a local reduction.

Blood coagulation

The fibrinmonomer (FM), soluble fibrin, is a good marker for a generalized blood coagulation in vivo. FM was increased in all groups 30 minutes after kallikrein injection, indicating increased disseminated coagulation. The inability to show reduced blood coagulation 30 minutes after kallikrein injection and only 90 minutes after completed infusion of lipid emulsions may be explained by the remaining phospholipid vesicles in the blood (Osterud et al, 1990). The reduction in coagulability is however seen after oral (Osterud et al, 1990) administration of marine oil or 1 to 2 days after intravenously infused marine oil emulsion.

Fibrinolytic variables

Tissue plasminogen activator (t-PA) is released from vascular endothelial cells after various types of stimuli. t-PA activates plasminogen to plasmin with fibrinolytic activity. An increase in t-PA was seen after kallikrein injection, with a maximum at 90 min. This increase was seen after infusion of MO-em in both doses but not after Intralipid.RTM.. During the pre-Kallikrein period after infusion of MO-em in high dose t-PA was increased to a level higher than the other groups, indicating stimulation of t-PA release, resulting in incresed fibrinolysis. This was not seen after infusion of Intralipid.RTM.. At 180 minutes post-kallikrein the levels were normalised in all groups, see FIG. 2.

Antiplasmin which inactivates plasmin, was reduced 180 minutes after kallikrein injection, see FIG. 3. This reduction was similar in the MO-emulsion and the placebo groups. However, in the group which received a high dose MO-emulsion, antiplasmin was reduced more markedly, reflecting an increased plasmin generation and a more prominent fibrinolytic response. During the pre-kallikrein period, antiplasmin was reduced in all groups, indicating increased fibrinolysis, probably mainly due to surgery.

Fibrinogen which is converted to fibrin by the action of thrombin, was reduced in the control and MO-em groups during the pre-kallikrein period, presumably caused by the surgical trauma, see FIG. 4. The level remained low also after kallikrein injection. At 180 minutes post-kallikrein the level of fibrinogen was lower in the FO-H group than in the other group.

Histopathology

The number of pigs examined in each group was: two controls, four high dose MO-em, four high dose Intralipid.RTM., three low dose MO-em and three low dose Intralipid.RTM..

There was presence of fibrin-like material in small blood vessels in the heart in one of the two control pigs and in one of four pigs given the high dose and one of three pigs given the low dose, respectively, of Intralipid.RTM.. The deposition of fibrin-like material was slight in all three cases. Deposition of fibrin-like material was not observed in any pig given the MO-em.

To observe microthrombi formation in the circulation by light microscopy after only three hours of induction of DIC may be difficult and probably explain the relatively slight morphological manifestation of DIC in this study. MSB and PTAH stains are recommended in the diagnosis of DIC (Hamilton et al, J Clin Path, 31, 609-619, 1978; Skorten, Acta Path Microbiol Scand, 61, 405-414, 1964) although immunological methods are more specific. A few spontaneous changes were recorded in some pigs but were considered to be without importance for the evaluation of the effect of treatment of lipid emulsion in DIC.

In conclusion, no evident light microscopically visible morphological manifestations of fibrin depositions in the tissues examined from the MO-em treated pigs could be observed.

Cardiovascular Parameters

1. Observations during the kallikrein infusion:

All animals had a fast rise in PAP, see FIG. 5, during the kallikrein infusion. The increase in PAP was less pronounced among the MO-em pretreated animals. All animals did also show a decrease in BP. The most pronounced decrease in BP was seen in the high MO-em pretreated animals. No major changes were observed in HR, WP, CO or PaO.sub.2 during this period.

2. Observations during the 180 minute post-Kallikrein injection period:

BP was restored to approximately the preinfusion values after about 30 minutes. PAP did also show a decline and the value was almost back to preinfusion level after about 180 minutes. Only minor changes were seen in wedge pressure, cardiac output and PaO.sub.2 during this period.

EXAMPLE 3

Need of Antioxidants

Syndromes with DIC may include radical reactions. Oxygen and hydroxy radicals may induce lipid peroxidation of PUFA in cell membranes which as a consequence may lead to cell damage and induction of the cascade systems. Therefore it is important to protect the PUFA in the emulsion, during the administration and after incorporation into biological membranes. The present experiment describes the evaluation of the need of antioxidants to the marine oil emulsions.

Marine oil emulsions (MO-em) (see Example 1) were infused intravenously 20 hours/day to rats over 14 days. The MO-em:s differed in the type of antioxidant added. The daily dose was 25 ml (5 g TG)/kg body weight (b.w.) and the experimental groups were: A) MO-em without antioxidant;, B) MO-em with a-tocopherol (vitamin E), 1 mg/g MO; C) MO-em with a-tocopherol, 1 mg/g MO, and vitamin C, 5 mg/g MO; D) MO-em with a-tocopherol, 5 mg/g MO; E) Intralipid.RTM. 20%; F) Physiological saline.

The results were the following;

1) Body weight, weight gain and organ weights (liver, spleen, kidney, lung, myocard, thymus) were similar in all groups.

2) The plasma level of vitamin E was lower in Group A but higher in Group D than in Groups E and F. The level of vitamin C in plasma did not

change.

3) The level in the liver of malondialdehyde, MDA, a marker of lipid peroxidation, was higher in Group A compared to all other groups.

4) Histopathological changes consisted mainly of fatty changes in the liver. In Groups A-D these changes were more evident in the Kupffer cells than in the hepatocytes contrary to the findings in Group E. The granulomatous reaction in the liver was more pronounced in Group D than in the other groups.

EXAMPLE 4

Dose-Response Sturdy

Marine oil containing a high degree of .**omega.3-fatty acids** has anti-thrombotic and anti-**inflammatory** effects and effects on hemostasis and immune defence. In order to facilitate a more specific use of the .**omega.3-fatty acids** it is important to have information about the dose-response relationships for the various effects and possible side effects. The aim of this study was to evaluate the dose-response relationship for effects of the .**omega.3-fatty acids** on fatty acid incorporation, eicosanoid level (**inflammation**), hemostasis, immune defence and safety. Dose-response relationships for biologcial effects induced by marine oil (MO)-emulsions, containing different amounts of marine oil triglycerides, (see Example 1) were evaluated.Three different 20% MO-emulsions, of which the composition of the oil was 100% (w/w)MO (Group A); 50% MO+50% soybean oil (SBO) (Group B); 10% MO+90% SBO (Group C) were infused intravenously to rats. The effects were compared with those induced by Intralipid.RTM. 20% (Group E) and physiological saline (Group F). The daily dose, 25 ml (5 g TG)/kg body weight (b w) in all groups was infused during 20 hours/day to rats during 14 consecutive days. All MO-emulsions contained .alpha.-tocopherol, 1 mg/g oil.

The results were as follows:

1) EPA and DHA in liver and spleen lipids increased dose-dependently in Groups A-C, whereas arachidonic and linoleic acids decreased, compared to Group F. Intralipid.RTM. (Group E) induced the opposite changes in these fatty acids. The fatty acid pattern was "normalized" by a low dose of MO-emulsion (Group C).

2) Red blood cell viscosity was reduced to a similar degree by the different MO-emulsions in Groups A-C.

3) The level of thromboxanes, which are prothrombotic in blood, was dose-dependently reduced by the different MO-emulsions with the threshold level in Group C.

4) MO-emulsion in the highest dose was more immunosuppressive than Intralipid.RTM.. Proliferation of splenic cells and thymocytes (3H-thymidin incorporation after Con A stimulation) was depressed more in group A, but similarly in Groups B and C, compared to Group E.

5) Final body weight, weight gain and the relative weights (g/kg b.w.) of liver, spleen and kidney were slightly higher in Groups A and B than in Groups E and F.

6) The degree and nature of fatty changes in Group C were similar to, whereas those in Groups A and B were somewhat more pronounced than those

in Group E. The histopathological changes were considered to be slight and probably of no clinical relevance.

7) The mount of lipids in liver and spleen was similar in all groups.

8) The level of malondialdehyde, MDA, a marker of lipid peroxidation, was lower in Groups C and E and similar in Groups A and B to those in Group F.

9) The level in blood of vitamin E (.alpha.-tocopherol) was similar in Groups A-C to that in Groups E and F.

The results show that a relatively low dose of MO-emulsion can be chosen to increase the membranal content of **.omega.3-fatty acids** and reduce the RBC viscosity, leading to improved nutritive circulation, without risking the less desirable ones (immuno-suppression, histopathological changes, lipid peroxidation). A somewhat higher dose is needed for reduction of the level of thromboxanes.

SUMMARY

DIC is still frequently seen in combination with major trauma, septicemia, meningoencephalitis and pancreatitis.There is often however a 12-26 hours elapse between the onset of symptoms and the debut of coagulation and respiratory distress. Major efforts are today spent on therapeutic manoeuvres in order to prevent these disorders from appearing; early fracture stabilisation, antibiotics, pain management, stress reduction and the goal is also to optimize oxygen delivery. Prophylactic heparin therapy is also sometimes given. Still these syndromes appears in a non-systematic fashion and with a high frequency of most complicated course. Therefore it is most interesting, if some therapy could be given in advance to most patients at risk, with the potential effect of lowering the incidence of DIC.

We have now found that preparations containing **.omega.3-fatty acids** have advantageous effects in the treatment of DIC, which may be a symptom as such or included in other symptoms such as sepsis or ischemia. The effects of **.omega.3-fatty acids** are seen very early, already after two hours of infusion, and can thus be of value to reduce the incidence of, and as a treatment of the DIC syndrome which is mostly an acute situation. The effects of MO-emulsion in comparison to the control and Intralipid.RTM. are summarised in the table below.

TABLE 1

Positive effects of Mo-em in comparison to the control and to Intralipid .RTM.

Effect of KK in the Para-meter	At time (min) post - control group	KK FO-H	Effects of Marine oil emulsion in comp. to placebo FO-L	in comp. to Intralipid .RTM. FO-H	FO-L	Positive effects of MO
PAP	.uparw.	5-30	.dwnarw.	.dwnarw.	.dwnarw.	.dwnarw. Reduced vascular resistance

TPA .uparw. 0-90 .uparw.
.uparw.
.uparw.
Increased
fibrinolysis
APL .dwnarw.
180 .dwnarw.
.apprxeq.
.dwnarw.
.dwnarw.
Increased
fibrinolysis
FBG .dwnarw.
0-180 .dwnarw.
.apprxeq.
.dwnarw.
.dwnarw.
Reduced
vulnerab. to
PL.AG.
-- 0.sup.1 .dwnarw.
.dwnarw.
.dwnarw.
.dwnarw.
Antithrombotic
effect
FIBRIN
.uparw. 180 .dwnarw.
.dwnarw.
.dwnarw.
.dwnarw.
Reduced DIC
DE- symptomes
POSITS

.sup.1 post. emulsion inf. (0'): .dwnarw. lower; .uparw. higher; .apprxeq similar; -- no effect

An improvement of the DIC-syndrome was seen already after a two hours infusion of marine oil emulsion. The positive effects on hemostasis and DIC are obtained after a short single infusion, which together with the anti-**inflammatory** effects expected after repeated infusion may have wide implications on DIC related syndromes.

After two hours of infusion of marine oil emulsion the increase in PAP, induced by kallikrein-DIC, was reduced, indicating reduced ventilatory complications associated with DIC in the lungs. The reduction of the kallikrein induced increase in PAP and the reduction of MAP seen after infusion of MO-em indicates vasodilation induced by .**omega**.
3-fatty acids . This effect may be explained either by reduced local production of TXA2 or induction of the release of NO. The results indicate that a short term infusion of a high dose of MO-em concomitant to an increase in plasma kallikrein and induction of DIC induces release of NO. The fibrinolytic response was increased and no fibrin deposits could be found in any of the organs tested (kidney, lung, heart and spleen). These end-point effects (reduced PAP, increased fibrinolysis, no fibrin deposits) show that the magnitude of the induced DIC symptoms was reduced. The tendency of the platelets to aggregate was minimized, indicating reduced risk of thrombosis. This effect may be explained by reduced TXA2 production in platelets or stimulated release of NO in endothelial cells. The reduced level of fibrinogen, seen after only two hours of infusion of marine oil emulsion, may together with

other positive effects shown for .**omega**.3-**fatty acids** minimize the vulnerability for heart attacks. PaO.sub.2 was maintained. The reduction in RBC viscosity suggests increased nutritional blood flow through capillaries.The positive effects on hemostasis are obtained before, and with a lower dose than that needed to suppress the immune defence. Thus, by a short-term high dose of marine oil emulsion or long-term dosing of a mixture of marine oil and a vegetable oil in emulsion, the risk for immunosuppression is minimized. The effects seen after infusion of MO-em were dose-related and positive in comparison to the changes induced by kallikrein injection in the control and Intralipid.RTM. groups.

We have also shown that the addition of dl-.alpha.-tocopherol, 1 mg/g oil, is enough to counteract the peroxidation of the highly polyunsaturated fatty acids from marine oil and the reduction of endogenous .alpha.-tocopherol and vitamin C induced by MO-em. Furthermore, we have shown that the positive effects of .**omega**.**3-fatty acids** on DIC were obtained when administered together with an antioxidant, .alpha.-tocopherol. From these results it may be concluded that the MO-em studied, containing dl-.alpha.-tocopherol, 1 mg/g oil, does not induce lipid peroxidation and is efficacious on DIC.

The positive effects of .**omega**.**3-fatty acids** on DIC are obtained after a single dose and before a reduction on the eicosanoid level is obvious. Thus, a low dose can be expected to be effective during long term treatment If the dose is increased a reduction in eicosanoid synthesis and thereby the anti-**inflammatory** effect is obtained.This anti-**inflammatory** effect is in favour over that obtained with NSAID:s, since these drugs block only cyclooxygenase and thereby provide more substrate for the lipoxygenase pathway, leading to enhanced monocyte activation as expressed by increased lipoxygenase effects and induced tissue factor activity. A concomitant vasodilation and increase in nutritional blood flow, reduction in PAP, and maintained PaO.sub.2, is a great and unexpected advantage compared to present treatment with vasodilators, which usually reduce PaO.sub.2. Marine oil emulsions or other preparations containing .**omega**.**3-fatty acids** can thus be therapeutically useful to treat severe tram and to help to prevent the development into various forms of DIC. Such emulsions can also be useful nutritionally for example to patients with DIC, or to patients vulnerable to the development of DIC, who also need TPN or in long term TPN, to reduce the symptoms of or to help to prevent the development into more chronic forms of DIC.

ABBREVIATION LIST

AA	Arachidonic acid
B.w.	Body weight
BP	Blood pressure
CO	Cardiac output
ConA	Concanavalin A
DHA	Docosahexaenoic acid
DIC	Disseminated intravascular coagulation
EDRF	Entothelial derived relaxing factor
EPA	Eicosapentaenoic aicd
FM	Fibrin monomer
HE	Haematoxylin-eosin
IL-I	Interleukin I
IL	Intralipid .RTM.
IL-H	Intralipid, high dose
IL-L	Intralipid, low dose
KK	Kallikrein

LPS	Lipopolysacharides
LVP	Left ventricular pressure
MAP	Mean arterial pressure
MDA	Malondialdehyde
MO	Marine oil
MO-em	Marine oil emulsion
MO-H	Marine oil emulsion, high dose
MO-L	Marine oil emulsion, low dose
MOF	Multiorgan failure
MSB	Martius scarlet blue
MUM-PGI	Main urinary metabolit of prostacyclin
MUM-TXA	Main urinary metabolit of thromboxane
NO	Nitric oxide
NSAID	Nonsteroidal anti-**inflammatory** drugs
PaO2	Arterial pressure of oxygen
PAP	Pulmonary artery pressure
PCWP	Pulmonary capillary wedge pressure
PTAH	Phosphotungstic acid haematoxylin
PUFA	Polyunsaturated fatty acids
RBC	Red blood cells
t-PA	Tissue plasminogen activator
TF	Tissue factor
TG	Triglycerides
TNF	Tumor necrotic factor
TPN	Total parenteral nutrition
WP	Wedge pressure

CLM What is claimed is:

1. Method of treating or preventing a pathological increase in pulmonary arterial pressure (PAP) in a patient in need thereof said PAP being induced by vasoactive mediators not involving thromboxane A2, which comprises administering to said patient in need thereof a preparation containing an effective amount of **.omega.3-fatty acid**, a salt or a derivative thereof in order to affect vasoactive mediators other than those involving thromboxane A2.

2. Method according to claim 1 wherein said **.omega.3-fatty acid**, salt or derivative thereof is derived from marine oil or from vegetable oil or from phospholipids.

3. Method according to claim 1 wherein said preparation is in the form of an aerosol for inhalation, a dosage form for nasal application or in a dosage form for oral or peroral administration.

4. Method according to claim 1 wherein said preparation also contains an antioxidant.

5. Method according to claim 1 wherein said **.omega.3-fatty acid** is selected from the group consisting EPA, DHA, salt thereof, derivative thereof and mixtures thereof.

6. Method according to claim 1 wherein said preparation is in the form of a tablet or capsule for oral or peroral administration.

7. Method according to claim 1 wherein the preparation containing **.omega.3-fatty acid** is adapted for total parenteral nutrition and optionally comprises **.omega.6-fatty acid**.

8. Method according to claim 1 wherein said preparation is adapted for long-term treatment.

9. The method of claim 1 wherein said preparation is an emulsion

comprising 0.5-50% (w/v) of an oil; 0.1-80% (w/v) of a phospholipid, at least 0.5% (w/v) of said **.omega.3-fatty acid** or salt or derivative thereof.

10. The method of claim 9 wherein said oil is present in an amount of 5-30% (w/v) and said phospholipid is present in an amount of 0.1-20% (w/v).

11. The method of claim 4 wherein said antioxidant is selected from the group consisting of .alpha.-tocopherol, vitamin C, a carotenoid and a retinoid.

12. The method of claim 1 wherein said preparation is administered intravenously.

INCL INCLM: 514/549.000
INCLS: 514/078.000; 514/458.000; 514/474.000; 514/560.000; 514/824.000
NCL NCLM: 514/549.000
NCLS: 514/078.000; 514/458.000; 514/474.000; 514/560.000; 514/824.000
IC [6]
ICM: A61K031-22
EXF 514/549; 514/560; 514/824; 514/458; 514/474; 514/78
ARTU 125
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 6 OF 33 USPATFULL
AN 1998:22206 USPATFULL
TI Enteral formulation designed for optimized nutrient absorption and wound
healing
IN Gray, Debora, Evanston, IL, United States
Schmelkin, Nancy S., Buffalo Grove, IL, United States
Alexander, John, Kenilworth, IL, United States
Mark, David A., Oak Park, IL, United States
Twyman, Diana, Chicago, IL, United States
PA Nestec Ltd., Vevey, Switzerland (non-U.S. corporation)
PI US 5723446 19980303 <--
AI US 1996-680703 19960717 (8)
RLI Continuation of Ser. No. US 1993-172857, filed on 23 Dec 1993, now abandoned
DT Utility
FS Granted

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EXNAM Primary Examiner: Tsang, Cecilia J.; Assistant Examiner: Mohamed, Abdel A.
LREP Hill, Steadman & Simpson
CLMN Number of Claims: 26
ECL Exemplary Claim: 1
DRWN No Drawings

AB The present invention provides an enteral nutritional formulation that meets the nutrient requirements of intensive care patients who may have compromised absorption capacity. The present invention meets the unique nutrient needs of the patient that are generated due to tissue repair and healing requirements. To this end, in an embodiment the present invention provides a method for treating and/or providing nutritional support to intensive care patients comprising the steps of administering a therapeutically effective amount of a composition comprising: a protein source; a carbohydrate source; and a lipid source including a source of medium chain triglycerides, a source of **omega-3 fatty acids**, and a source of **omega-6 fatty acids**.

PARN This is a continuation of application Ser. No. 08/172,857, filed Dec. 23, 1993 now abandoned.

SUMM BACKGROUND OF THE INVENTION

The present invention relates to nutritionally fortified pharmaceutical compositions. More specifically, the present invention relates to compositions for use in intensive care patients.

Intensive care patients describe a broad population of patients who may suffer from a variety of diseases or insults. These patients, however, exhibit some similar requirements. For example, patients suffering from traumatic injury, burns, post-surgery, and some disease states have a significant need for increased nutrients and energy as compared to individuals who are not challenged by such metabolic stress.

Indeed, non-essential nutrients and substances that a body typically can

synthesize in adequate supply, may become limiting. Additionally, absorption of nutrients from the gut can be compromised even when there is no direct injury to the gastrointestinal system.

Many intensive care patients are fed either with parenteral formulations or enteral formulations either to replace or supplement a typical diet. For example, in 1991, of an estimated 2.4 million trauma patients in the United States, 13% (310,000) required nutrition support beyond food. Of these patients, 62% of the patients were supported using enteral nutrition, 70% tube-feeding, and 30% oral supplements, while 38% were initially supported using parenteral nutrition and progressed to tube-feeding, if they survived. Similarly, of about 106,000 burn patient admissions in 1991 in the U.S., approximately 20% (21,000) required nutritional support. Of this group, 95% were started on enteral nutrition, 70% began on tube feeding and 30% started on oral supplements.

Numerous enteral formulations have been targeted for trauma and burn patients. These products include: Mead-Johnson's TRAUMACAL.RTM.; Sandoz's IMPACT.RTM.; Abbott Laboratories' ALITRAQ.RTM.; and McGaw's IMMUN-AID.RTM..

Although such products are used in an attempt to treat and/or provide nutritional requirements for such patients, the inventors of the present invention do not believe that these products meet the needs of such patients.

Accordingly, there is a need for an enteral nutritional formulation which meets the nutrient requirements of intensive care patients who may have altered nutritional requirements and compromised absorptive capacity.

SUMMARY OF THE INVENTION

The present invention provides an enteral nutritional formulation that meets the nutrient requirements of intensive care patients who may have compromised absorption capacity. The present invention meets the unique nutrient needs of the patient that are generated due to tissue repair and healing requirements.

To this end, in an embodiment the present invention provides a method for treating and/or providing nutritional support to intensive care patients comprising the steps of administering a therapeutically effective amount of a composition comprising: a protein source; a carbohydrate source; and a lipid source including a source of medium chain triglycerides (MCTs), a source of **omega-3 fatty acids**, and a source of **omega-6 fatty acids**. In an embodiment, the source of **omega-3 fatty acids** comprises at least 2.3% of the total calories.

In an embodiment, a method for treating and/or providing nutritional support to an intensive care patient is provided comprising administering a therapeutically effective amount of a composition comprising: a high protein content of at least 22% of the total calories; a carbohydrate source; and a high lipid content of at least 30% of the total calories.

In an embodiment, a method for treating an intensive care patient is provided comprising administering a therapeutically effective amount of a composition comprising: 22-28% of the calories as a protein; 33-45% of

the calories as a lipid, the lipid provides at least 40% of its caloric content as medium chain triglycerides, and further including an **omega-3 fatty acid** source and an **omega-6 fatty acid** source; and a carbohydrate source. Preferably, the caloric density of the composition is at least 1.3 Kcal/ml.

If desired the composition can include sources of: glutamine; arginine; proline; and/or cysteine.

It is an advantage of the present invention that it provides an enteral nutritional formulation that is designed to optimize nutrient absorption and wound healing in trauma patients.

Moreover, an advantage of the present invention is to provide a composition having a high protein content, a high lipid content, and a high caloric density to meet protein and energy needs.

Furthermore, an advantage of the present invention is to provide a composition that has reduced water and carbohydrate content reducing the risk of diarrhea due to carbohydrate intolerance, hyperglycemia, over hydration, and the like.

Still further, an advantage of the present invention is that nutrient malabsorption is reduced by the absence of whole proteins and by the use of protein hydrolysate, free amino acids and medium chain triglycerides in the enteral formulation of the present invention.

Additionally, an advantage of the present invention is that it is a ready-to-use formulation, and not a powder that requires mixing before use, reducing the risk of bacterial contamination during the mixing process.

Moreover, pursuant to the present invention, healing and tissue repair/cell division is promoted by the use of certain components.

It is also an advantage of the present invention that **inflammatory** reactions are minimized.

Additional features and advantages of the present invention are described in, and will be apparent from, the detailed description of the presently preferred embodiments.

DETD DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

The present invention provides enteral formulations specifically designed for use with intensive care patients, specifically, trauma, burn, and post-surgery patients. Moreover, the present invention provides methods of treating such patients.

Pursuant to the present invention, an enteral formulation is provided that is designed to optimize nutrient absorption and wound healing in trauma patients. The enteral formulation of the present invention meets the nutrient requirements of such patients with compromised absorptive capacity. The formulation also meets nutrient needs unique to tissue repair and healing of the patients.

Generally, pursuant to the present invention, a ready-to-use enteral formulation is provided. The formulation can provide the total nutritional requirements of the intensive care patient or can act as a supplement. The product is designed preferably to be fed to the patient

by tube. The product can be provided, for example, in cans or a spike and hang bag. The product is ready to use and does not require reconstitution or mixing prior to use.

In a preferred embodiment, the enteral formulation has a high caloric content. In an embodiment, preferably, the caloric content is between approximately 1.3 to about 1.5 Kcal/ml. It is necessary to provide a moderate-to-high caloric intake to spare protein. Caloric needs in severe trauma, burn, and post-surgical patients typically range from 25 to about 35 Kcal/Kg, e.g., 1800 to 2300 Kcal for a convalescing 70 Kg adult. In fact, severe burn patients can require even higher caloric needs.

Additionally, due to increased metabolic activity, such patients require high protein intake to reduce negative nitrogen balance and support wound repair. Protein needs average 2.0 g of protein Kg body weight or, e.g., 140 grams of protein per day for a convalescing 70 Kg adult. Therefore, the formulation has a high protein content, preferably at least approximately 22% of the calories of the product are provided as protein. In an embodiment, up to 28% of the calories are provided as protein.

A variety of proteins can be utilized. In an embodiment, the protein is hydrolyzed casein plus free amino acids. If desired, the protein source can be high in glutamine and perhaps in cysteine. In an embodiment, the protein source is enriched with arginine and proline as free amino acids.

The use of protein hydrolysate and free amino acids reduces the potential for nutrient malabsorption. Additionally, by providing a high glutamine, arginine, protein and/or cysteine content, wound healing and tissue repair/cell division is promoted.

In an embodiment, 25% of the total caloric content of the product is protein. In an embodiment, 80-85% of the protein will be partially hydrolyzed casein, 13-15% arginine and 4-6% proline. In an embodiment, 68-70% of the protein will be partially hydrolyzed casein, 17-20% will be partially hydrolyzed whey protein and 13-15% will be arginine. In a preferred embodiment, 85-88% of the protein will be partially hydrolyzed casein and 12-15% will be arginine. In choosing the protein source, the present invention maximizes the naturally available levels of desirable amino acids such as arginine, cysteine, proline and glutamine at the highest bioavailability and product stability.

The formulation of the present invention includes a lipid fraction. Preferably, approximately 33% to about 45% of the formulation, by calories, is provided as a lipid. In a preferred embodiment, 39% of the calories are provided as a lipid.

The lipid fraction contains significant amounts of omega-3 rich fatty acids and medium chain triglycerides. Preferably, the lipid fraction comprises approximately 40% to about 60%, by calories MCTs. MCTs are more easily absorbed and metabolized as compared to long chain triglycerides (LCTs). The use of MCT will reduce the risk of the potential for nutrient malabsorption. A low omega-6 content and a high omega-3 content are provided. Preferably, the ratio of omega-6 to **omega-3 fatty acids** is less than 2.0:1. The low omega-6: omega-3 ratio reduces the incidence and severity of **inflammatory** reactions. **Omega-3 fatty acids** may modulate the negative, immune-mediated reactions brought about by high omega-6 intake. Therefore, oil blends which contain omega-3 (or are, at a minimum, low in omega-6) are preferred.

Accordingly, in an embodiment, a fish oil rich in **omega-3 fatty acids** is preferred, as fish oils contain two longer chain length **omega-3 fatty acids**: eicosapentaenoic acid (EPA, C22:5, n3) and docosahexaenoic acid (DHA, C22:6, n3). Soy oil is also preferred, in that it contains approximately 7% **linolenic acid** (C18:3, n3), in order to insure that a safe minimum level of shorter length **omega-3 fatty acids** is delivered, and also contains approximately 50-55% linoleic acid (C18:2, n-6), in order to insure that a safe minimum level of **omega-6 fatty acids** is delivered (essential fatty acids). In an embodiment of the present invention, the lipid component comprises by weight 50% MCT, 25% fish oil and 25% soy oil (includes soy oil and soy lecithin).

In addition to the ability of omega-3 to modulate **inflammatory** reactions, likewise, the antioxidant vitamins and minerals also reduce the incidence of severity of **inflammatory** reactions.

By utilizing a formulation having high protein and fat content, protein and energy requirements are met. However, at the same time, pursuant to the present invention, the formulation includes reduced water and carbohydrate content. This reduces the risk of over hydration, hyperglycemia, and carbohydrate intolerance.

Preferably, the formulation is approximately 35% to about 40%, by calories, carbohydrates. By way of example, the carbohydrates can be chosen from maltodextrin, corn starch, sucrose, and corn syrup solids.

In an embodiment, the present invention includes soluble or insoluble fiber, and/or carob pod powder or extract that is rich in insoluble tannins. In an enteral product, especially one to be provided by tube feeding, this provides anti-diarrhea characteristics. Magnesium can be reduced below U.S. RDA levels (400 mg/day), further limiting the potential for tube-fed induced diarrhea. An example of the use of tannins to reduce the incidence of diarrhea is set forth in U.S. patent application Ser. No. 887,360 entitled: "ENTERAL FORMULATION DESIGNED TO REDUCE DIARRHEA IN TUBE-FED PATIENTS" now abandoned, the disclosure of which is hereby incorporated herein by reference.

Preferably, anti-oxidant vitamins and minerals are increased to above the U.S. RDAs. This will insure that the patient receives at least 100% of the U.S. RDA as well as insure that any additional micronutrients that are necessary due to the patient's state will be provided. The formulation, in an embodiment, will provide approximately 5-6 mg/1500 Kcal of beta-carotene. Beta-carotene is a precursor for Vitamin A and has some unique antioxidant properties.

Of course, it will be appreciated that a variety of formulations are possible in accordance with the present invention. An example of a formulation in accordance with the present invention includes a formulation having a caloric density of 1.5 Kcal/ml. This is equivalent to 375 Kcal/250 ml which will, in a preferred embodiment, by one unit (can or container) of product.

In this embodiment, preferably, protein comprises 25%, by calories, of the product. This is equivalent to 94 grams/liter. A variety of different components are possible for the protein portion of the product. In an embodiment, casein plus arginine can be utilized. In a further embodiment, casein plus arginine plus proline can be utilized for the protein component.

Preferably, in this embodiment, the lipid component comprises approximately 39% of the calories of the product. This will be equal to

approximately 65 grams/liter. In the embodiment, approximately 50% of the lipid component is MCTs and 25% of the lipid component is fish oil. Preferably, 19 to 21% of the lipid component is soy oil and 4-6% soy lecithin. This will provide an omega-6:omega-3 ratio of approximately 1.8:1.

Preferably, in this embodiment, the carbohydrates comprise 36% of the calories. This is equivalent to 135 g/l. In the embodiment, maltodextrin and corn starch are used.

The total calories/nitrogen in this embodiment is approximately 90:1. The total non-protein calories/grams of nitrogen is approximately 67:1. Osmolality will be less than or equal to 500 mOsm/kg H.sub.2 O. It is envisioned that the shelf-life of the product will be approximately 12 months.

Pursuant to the present invention, the **omega-3 fatty acids** as a percent of the total calories of the product will be greater than 2.3%. Anti-**inflammatory** activity is believed to be achieved at 2.2% to 3% of the calories of the product.

Anti-thrombotic and hypolipidemic is also believed to be a benefit of such high levels of omega-3. As set forth above, preferably, fish oil and soy oil are utilized. A number of potential beneficial effects are achieved by using fish oil.

Most typical nutritional products have less than 2.3% of the total calories as **omega-3 fatty acids**.

To this end, the following commercial available products have the following **omega-3 fatty acid** content (as a % of total calories): IMPACT.RTM. 1.6%; IMMUN-AID.RTM. 1.0%; PEPTAMEN.RTM. VHP 1%; Promote 0.9%; TRAUMACAL.RTM. 0.3%; and PEPTAMEN.RTM. 0.2%.

In an embodiment of the present invention, the formulation of the present invention includes at least 3% of the total calories as arginine. Enhanced wound healing with arginine is believed to be provided at quantities greater than 3% of the total calories.

Additionally, in an embodiment, the present invention includes significant amounts of proline. In an embodiment, the proline content is at least 2.0% of the total calories. Proline content as a percent of specific proteins is as follows: gelatin=16.1%; casein=9.6%; whey=5.7%; and soy=5.4%.

Additionally, in an embodiment, the present invention can include significant amounts of cysteine. In an embodiment, the present invention only provides approximately 0.6% of the total calories as cysteine. This is substantially in line with ad-libitum diets. However, the present invention, in an embodiment, provides 0.17% of the total calories as cysteine. Cysteine content of various proteins is as follows: casein=0.3%; total milk products=0.9%; soy protein=1.2%; whey protein=2.0%; and egg white protein=2.5%.

Pursuant to the present invention, non-protein calories/grams of nitrogen (NPC/gN) is determined so as to provide a composition that spares the use of proteins as the calorie source. Patients with severe metabolic stress (trauma, burns) preferably should receive a product with an NPC/gN of less than 100:1 because of their increased protein requirements. Pursuant to the present invention, the formulation provides compositions having less than or equal to 70:1. The weight/nitrogen weight of certain proteins is as follows: arginine

3.11:1; glutamine 5.21:1; casein 6.25:1; protein 6.25:1; whey 6.38:1; proline 8.21:1; branched chain amino acids 8.79:1.

By way of example, and not limitation, examples of formulations of the present invention will now be given.

FORMULA EXAMPLE NO. 1

A liquid, ready-to-use enteral product with protein at 25% of total calories: 87% from partially hydrolyzed casein and 13% from the free amino acid arginine. Carbohydrates would be 35-40% of calories. Lipids comprise 38-42% of calories, preferably a blend of medium chain triglycerides (50%), fish oil (25%), soy oil and soy lecithin (25% total of both soys). Vitamin and mineral content would meet preferably daily requirements in 1500 calories.

FORMULA EXAMPLE NO. 2

A liquid, ready-to-use enteral product with protein at 25% of total calories: 60% from partially hydrolyzed casein, 20% from partially hydrolyzed whey protein, 15% from the free amino acid arginine and 5% from the free amino acid proline. Carbohydrates would be 35-40% of calories. Lipids comprise 38-42% of calories, preferably a blend of medium chain triglycerides (50%), fish oil (25%), soy oil and soy lecithin (25% total of both soys). Vitamin and mineral content would meet preferably daily requirements in 1500 calories.

By way of example, and not limitation, contemplative examples of the use of the present invention will now be given.

CONTEMPLATIVE EXAMPLE NO. 1

Two hundred patients admitted to intensive care units with moderate to severe trauma are nutritionally supported by the use of tube-fed enteral formulas. Half receive a whole protein based product at 1.0 calories/mL, with protein as 22% of calories (a combination of whole protein and free amino acid arginine), carbohydrates at 50-55% of calories and lipids at 20-25% of calories, with 25% as MCT and the remainder fish oil and sunflower oil. Vitamin and mineral U.S. RDAs met in 1500 calories (1500 mL). Half receive a formula described in this invention: a liquid, ready-to-use enteral product at 1.3-1.5 Kcal/mL with protein at 25% of total calories (87% from partially hydrolyzed casein and 13% from the free amino acid arginine), carbohydrates at 35-40% calories and lipids at 38-42% of calories, with half of the lipid as MCT, 25% fish oil and 25% soy oil and soy lecithin. Vitamin and mineral U.S. RDAs met in 1500 calories (1000 mL).

Many of the patients receiving the whole protein diet were unable to receive the recommended calorie and protein intakes of 2200-2500 calories and 140 grams protein because of intolerance and diarrhea and conflicts with the need to not overhydrate. By comparison, the elemental and calorically dense product described in this invention, it is believed, will be able to deliver 2250 calories and 140 grams protein in 1.5 liters/day with a minimal incidence of intolerance or diarrhea. When using APACHE scoring to predict outcomes, the patients fed the enteral diet described in this invention will, it is believed, have a shorter average length of stay and fewer **inflammatory** complications than would have been expected based on experiences with whole

protein-based diets which contain less than 2.3% of calories as a mixture of **omega-3 fatty acids** (linolenic, EPA and DHA).

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

CLM What is claimed is:

1. A method for providing nutrition to a trauma, burn or post-surgery patient comprising the step of enterally administering to the patient a therapeutically effective amount of a composition comprising: a protein source comprising at least 25% of the total calories; a carbohydrate source comprising approximately 35% to about 40% of the total calories; and a lipid source comprising approximately 33% to about 45% of the total calories including a source of medium chain triglycerides comprising approximately 40% to about 60% of the lipid source, a source of **omega-3 fatty acids**, and a source of **omega-6 fatty acids**.

2. The method of claim 1 wherein the omega-3 source provides approximately 2.2% to about 3% of the total calories.

3. The method of claim 1 wherein the composition provides a source of arginine.

4. The method of claim 1 wherein the composition provides a source of proline.

5. The method of claim 1 wherein the protein source includes a majority of the protein calories as partially hydrolyzed proteins and does not contain whole proteins.

6. The method of claim 1 wherein the formulation is fed through a tube to the patient.

7. The method of claim 1 wherein the composition includes a source of beta-carotene.

8. A method for providing nutrition to a trauma, burn or post-surgery patient comprising the step of enterally administering to the patient a therapeutically effective amount of a composition comprising: a high protein source comprising approximately 22% to about 28% of the total calories, the protein source includes a majority of the protein calories as partially hydrolyzed proteins and does not contain whole proteins; a carbohydrate source of approximately 35% to about 40% of the total calories; and a lipid source of approximately 33% to about 45% of the total calories, the lipid source comprising at least 40%, by calories, medium chain triglycerides.

9. The method of claim 8 wherein approximately 2.2% to about 3% of the total calories are provided by **omega-3 fatty acids**.

10. The method of claim 8 wherein the composition provides a source of arginine.

11. The method of claim 8 wherein the composition provides a source of proline.

12. The method of claim 8 wherein the composition includes a source of

beta-carotene.

13. A method for providing nutrition to a trauma, burn or post-surgery patient comprising the step of enterally administering a therapeutically effective amount of a composition comprising: approximately 22% to about 28% of the total calories as protein; approximately 33% to about 45% of the total calories as a lipid including a source of medium chain triglycerides and an **omega-3 fatty acid** source providing at least 2.3% of the total calories; approximately 35% to about 40% of the total calories as a carbohydrate source; and the composition having a caloric density of approximately 1.3 to about 1.5 Kcal/ml.

14. The method of claim 13 wherein the composition provides a source of arginine.

15. The method of claim 13 wherein the composition provides a source of proline.

16. The method of claim 13 wherein the protein source includes a majority of the total calories as partially hydrolyzed proteins.

17. The method of claim 13 wherein the lipid source includes approximately 40% to about 60% of the lipid calories as medium chain triglycerides.

18. The method of claim 13 wherein the composition includes a source of beta-carotene.

19. A composition for providing nutrition to a trauma, burn or post-surgery patient comprising: approximately 22-28% of the calories as a protein source, the protein source including approximately 68% to about 88% partially hydrolyzed protein; approximately 35-45% of the calories as a lipid including approximately 40% to about 60% as medium chain triglycerides, an **omega-3 fatty acid** source, and an **omega-6 fatty acid** source; approximately 35% to about 40% of the calories as a carbohydrate source; and the caloric density of the composition being approximately 1.3 to about 1.5 Kcal/ml.

20. The composition of claim 19 wherein the composition includes a source of arginine.

21. The composition of claim 19 wherein the composition includes a source of proline.

22. The composition of claim 19 wherein the composition includes a source of cysteine.

23. The composition of claim 19 wherein the composition includes a source of beta-carotene.

24. The composition of claim 19 wherein the protein source includes approximately 80% to about 85% partially hydrolyzed protein.

25. A composition for providing nutrition to a trauma, burn or post-surgery patient comprising: approximately 22-28% of the calories as a protein; approximately 35-45% of the calories as a lipid including approximately 40% to about 60% as medium chain triglycerides, an **omega-3 fatty acid** source, and an **omega-6 fatty acid** source; approximately 35%-40% of the calories as a carbohydrate source; and

approximately 12% to about 15% of the protein calories being provided by arginine.

26. A composition for providing nutrition to a trauma, burn or post-surgery patient comprising: approximately 22-28% of the calories as a protein; approximately 35-45% of the calories as a lipid including approximately 40% to about 60% as medium chain triglycerides, an **omega-3 fatty acid** source and an **omega-6 fatty acid** source; approximately 35%-40% of the calories as a carbohydrate source; and approximately 4% to about 6%, by protein calories, of the composition being provided by proline.

INCL INCLM: 514/021.000
INCLS: 514/002.000; 514/023.000; 514/054.000; 514/538.000; 514/560.000; 514/094.300; 426/072.000; 426/607.000; 426/656.000; 426/658.000; 424/DIG.013
NCL NCLM: 514/021.000
NCLS: 424/DIG.013; 426/072.000; 426/607.000; 426/656.000; 426/658.000; 514/002.000; 514/023.000; 514/054.000; 514/538.000; 514/560.000; 514/943.000
IC [6]
ICM: A61K038-00
ICS: A23J001-00; A23G003-00
EXF 514/21.2; 514/23.54; 514/558; 514/560; 514/943; 426/72; 426/607; 426/656; 426/658; 424/DIG.13
ARTU 181
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 7 OF 33 USPATFULL
AN 1998:12014 USPATFULL
TI Enternal formulation designed for optimized nutrient absorption and wound healing
IN Gray, Debora, Chicago, IL, United States
Schmelkin, Nancy S., Buffalo Grove, IL, United States
Alexander, John, Kenilworth, IL, United States
Mark, David A., Oak Park, IL, United States
Twyman, Diana, Chicago, IL, United States
PA Nestec Ltd., Vevey, Switzerland (non-U.S. corporation)
PI US 5714472 19980203 <--
AI US 1995-530877 19950920 (8)
RLI Continuation-in-part of Ser. No. US 1993-172587, filed on 23 Dec 1993, now abandoned
DT Utility
FS Granted

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TraumaCal Document bearing Nos. B00384-B00385.
TraumaCal Label bearing No. B00441.
TraumaCal Brochure bearing Nos. B00567-B00570.

EXNAM Primary Examiner: Tsang, Cecilia J.; Assistant Examiner: Mohamed, Abdel A.
LREP Hill, Steadman & Simpson
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN No Drawings

AB The present invention provides an enteral nutritional formulation that meets the nutrient requirements of intensive care patients who may have compromised absorption capacity. The present invention meets the unique nutrient needs of the patient that are generated due to tissue repair and healing requirements. To this end, in an embodiment the present invention provides a method for providing nutritional support to intensive care patients comprising the steps of administering a therapeutically effective amount of a composition comprising: a protein source; a carbohydrate source; and a lipid source including a source of medium chain triglycerides, a source of **omega-3 fatty acids**, and a source of **omega-6 fatty acids**.

PARN This application is a continuation-in-part of U.S. application Ser. No. 08/172,857 entitled: "ENTERAL FORMULATION DESIGNED FOR OPTIMIZED NUTRIENT ABSORPTION AND WOUND HEALING" filed Dec. 23, 1993, now abandoned.

SUMM BACKGROUND OF THE INVENTION

The present invention relates to nutritionally fortified pharmaceutical compositions. More specifically, the present invention relates to compositions for use in intensive care patients.

Intensive care patients describe a broad population of patients who may suffer from a variety of diseases or insults. These patients, however, exhibit some similar requirements. For example, patients suffering from traumatic injury, burns, post-surgery, and some disease states have a significant need for increased nutrients and energy as compared to individuals who are not challenged by such metabolic stress. Indeed, non-essential nutrients and substances that a body typically can synthesize in adequate supply may become limiting. Additionally,

absorption of nutrients from the gut can be compromised even when no direct injury to the gastrointestinal system exists.

Many intensive care patients are fed either with parenteral formulations or enteral formulations either to replace or supplement a typical diet. For example, in 1991, of an estimated 2.4 million trauma patients in the United States, 13% (310,000) required nutrition support beyond food. Of these patients, 62% of the patients were supported using enteral nutrition, tube-feeding and 30% oral supplements, while 38% were initially supported using parenteral nutrition and progressed to tube-feeding, if they survived. Similarly, of about 106,000 burn patient admissions in 1991 in the U.S., approximately 20% (21,000) required nutritional support. Of this group, 95% were started on enteral nutrition, 70% began on tube feeding and 30% started on oral supplements.

Numerous enteral formulations have been targeted for trauma and burn patients. These products include: Mead-Johnson's TRAUMACAL.RTM.; Sandoz's IMPACT.RTM.; Abbott Laboratories' ALITRAQ.RTM.; and McGaw's IMMUN-AID.RTM.. Although such products are used in an attempt to treat and/or provide nutritional requirements for such patients, the inventors of the present invention do not believe that these products meet the needs of such patients.

Accordingly, a need exists for an enteral nutritional formulation which meets the nutrient requirements of intensive care patients who may have altered nutritional requirements and compromised absorptive capacity.

SUMMARY OF THE INVENTION

The present invention provides an enteral nutritional formulation that meets the nutrient requirements of intensive care patients who may have compromised absorption capacity. The present invention meets the unique nutrient needs of the patient that are generated due to tissue repair and healing requirements.

To this end, in an embodiment, the present invention provides a method for providing nutritional support to intensive care patients comprising the step of administering a therapeutically effective amount of a composition. The composition preferably includes a protein source; a carbohydrate source; and a lipid source. The protein source is produced with the use of pancreatic enzymes, resulting in a unique peptide profile.

In an embodiment, a method for providing nutritional support to an intensive care patient is provided comprising administering a therapeutically effective amount of a composition with an improved protein source. The protein source contains a protein hydrolysate and free amino acids; the protein hydrolysate includes less than approximately 35%, by weight, peptides having a chain length of more than five amino acids.

In another embodiment, a method for providing nutritional support to a patient is provided that utilizes a composition containing approximately 80% to 85% of protein hydrolysate and approximately 15% to 20% of free amino acids. Preferably, the caloric density of the composition is at least 1.3 Kcal/ml.

In an embodiment, the protein hydrolysate includes less than approximately 35% by weight peptides having a chain length of more than five amino acids.

In yet another embodiment, the free amino acids of the composition comprise less than approximately 20% by weight of the protein source.

In an embodiment, the protein source comprises less than approximately 20% by weight peptides having a chain length of more than nine amino acids.

In another embodiment, the cysteine content of the protein source is at least approximately 0.25% of the total calories.

In an embodiment, a method for providing nutritional support to an intensive care patient is provided comprising administering a therapeutically effective amount of yet another composition. The composition comprises a protein source containing less than 20% peptides, by weight, having a chain length of more than nine amino acids.

If desired the composition can include sources of: arginine; proline; and/or cysteine. Sufficient cysteine is included to replenish the intracellular glutathione of the treated patient. Preferably, the composition contains at least 0.25% of its total calories from cysteine.

An advantage of the present invention is that it provides an enteral nutritional formulation that is designed to optimize nutrient absorption and wound healing in trauma patients.

Moreover, an advantage of the present invention is to provide a composition having a high protein content, a high lipid content, and a high caloric density to meet protein and energy needs.

Furthermore, an advantage of the present invention is to provide a composition that has reduced water and carbohydrate content, reducing the risk of diarrhea due to carbohydrate intolerance, hyperglycemia, over hydration, and the like.

Still further, an advantage of the present invention is that nutrient malabsorption is reduced by the absence of whole proteins and by the use of protein hydrolysate, free amino acids and medium chain triglycerides in the enteral formulation of the present invention.

Additionally, an advantage of the present invention is that it is a ready-to-use formulation, and not a powder that requires mixing before use, reducing the risk of bacterial contamination during the mixing process.

Moreover, pursuant to the present invention, the use of certain components promotes healing and tissue repair/cell division.

It is also an advantage of the present invention that **inflammatory** reactions are minimized.

Yet another advantage of the present invention is that it utilizes a composition containing a unique peptide profile. The peptide profile promotes improved tolerance, better absorption, superior nitrogen utilization and retention, more rapid repletion of gut integrity and a reduced length of stay for the patient.

Moreover, another advantage of the present invention is that it utilizes a protein hydrolysate that is produced with pancreatic enzymes, as opposed to microbial enzymes. Such pancreatic-derived peptides are more physiological than those produced with microbial enzymes.

Still further, an advantage of the present invention is that it provides an enteral product designed for intensive care patients that contains a high level of dipeptides and tripeptides. Peptide diets containing a high level of such peptides provides a multitude of benefits to the patient.

Additional features and advantages of the present invention are described in, and will be apparent from, the detailed description of the presently preferred embodiments.

DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

The present invention provides enteral formulations specifically designed for use with intensive care patients. For example, the present invention can be utilized to treat trauma, burn and post-surgery patients. Specifically, the present invention provides methods for providing nutritional support to such patients.

Pursuant to the present invention, an enteral formulation is provided that is designed to optimize nutrient absorption and wound healing in trauma patients. The enteral formulation of the present invention meets the nutrient requirements of such patients with compromised absorptive capacity. The formulation also meets nutrient needs unique to tissue repair and healing of the patients.

Generally, pursuant to the present invention, a ready-to-use enteral formulation is provided. The formulation can provide the total nutritional requirements of the intensive care patient or can act as a supplement. The product is designed preferably to be fed to the patient by tube. The product can be provided, for example, in cans or a spike and hang bag. The product is ready to use and does not require reconstitution or mixing prior to use.

In a preferred embodiment, the enteral formulation has a high caloric content. In an embodiment, preferably, the caloric content is between approximately 1.3 to about 1.5 Kcal/ml. Providing a moderate-to-high caloric intake is necessary to spare protein. Caloric needs in severe trauma, burn, and post-surgical patients typically range from 25 to about 35 Kcal/Kg, e.g., 1800 to 2500 Kcal for a convalescing 70 Kg adult. In fact, severe burn patients can require even higher caloric needs.

Additionally, due to increased metabolic activity, such patients require high protein intake to reduce negative nitrogen balance and support wound repair. Protein needs average 2.0 g of protein per Kg body weight or, e.g., 140 grams of protein per day for a convalescing 70 Kg adult. Therefore, the formulation has a high protein content, preferably at least approximately 22% of the calories of the product are provided as protein. In an embodiment, up to 28% of the calories are provided as protein.

Pursuant to the present invention, the protein source of the composition preferably includes a protein hydrolysate and free amino acids. The use of protein hydrolysate and free amino acids reduces the potential for nutrient malabsorption. A variety of hydrolyzed proteins can be utilized in the present invention. Suitable examples include casein hydrolysate and whey hydrolysate. Preferably, the protein source includes approximately 80% to 85% of protein hydrolysate and approximately 15% to 20% of free amino acids.

Pancreatic enzymes are preferably utilized to produce the protein hydrolysate of the present invention. The present invention is a peptide-based diet with high levels of di- and tripeptides. A variety of benefits arise from utilizing a protein source containing primarily dipeptides and tripeptides. For example, when compared to either a diet based on whole proteins or one containing primarily or solely free amino acids, the use of such peptide diets improves tolerance, promotes absorption, results in superior nitrogen utilization and retention, increases the repletion of gut integrity and possibly reduces the length of stay for a patient. Suitable hydrolysis methods that can be utilized to produce the protein hydrolysate of the present invention are described in various U.S. patents. For example, U.S. Pat. No. 4,427,658 entitled: "Total Enzymatic Hydrolysate From Whey Proteins and Process of Obtaining the Same," U.S. Pat. No. 4,358,465 entitled "Phosphopeptides From Casein-Based Material" (as well as the continuations and divisions therefrom, namely U.S. Pat. Nos. 4,495,176; 4,740,462; 4,816,398; 5,028,589) and U.S. Pat. No. 4,361,587 entitled: "Phosphopeptides From Casein-Based Material" (and related patents, such as U.S. Pat. No. 4,980,450) describe enzymatic hydrolysis by means of utilizing a proteolytic enzyme (e.g. pancreatin) that may be utilized to produce the protein hydrolysate of the present invention. The disclosure of these patents is hereby incorporated herein by reference. The pancreatic-derived peptides that are produced by such hydrolysis methods are generally more physiological than those produced by a microbial enzyme hydrolysis system. Thus, the present invention preferably includes such pancreatic-derived peptides.

The protein source of the present invention contains a unique peptide profile. In an embodiment, the protein hydrolysate in combination with free amino acids contains less than approximately 20% free amino acids, by weight, and less than approximately 20% peptides, by weight, with a chain length of more than nine amino acids. The hydrolysate of the present invention preferably contains less than approximately 35% peptides, by weight, with a chain-length of more than five amino acids.

As noted above, the protein source of the present invention includes a portion as free amino acids. In an embodiment, the protein source is enriched with arginine and proline as free amino acids. The hydrolysate source also preferably includes a sufficient amount of cysteine to replenish intracellular glutathione in the patient. Providing a high arginine, proline and/or cysteine content promotes wound healing and tissue repair/cell division.

In an embodiment, approximately 25% of the total caloric content of the product is protein. Pursuant to the present invention, either a single hydrolyzed protein or a combination of at least two hydrolyzed proteins can be utilized. For instance, in an embodiment, approximately 80% to 85% of the protein will be partially hydrolyzed casein, approximately 13% to 15% arginine and approximately 4% to 6% proline. In another embodiment, approximately 50% to 55% of the protein will be partially hydrolyzed casein, approximately 30% to 35% partially hydrolyzed whey protein and approximately 13% to 15% will be arginine. In choosing the protein source, the present invention maximizes the natural available levels of desirable amino acid such as arginine, cysteine, proline and glutamine at the highest bioavailability and product stability.

The formulation of the present invention also includes a lipid fraction.
Preferably, approximately 33% to about 45% of the formulation, by

calories, is provided as a lipid. In a preferred embodiment, 39% of the calories are provided as a lipid.

The lipid fraction contains significant amounts of omega-3 rich fatty acids and medium chain triglycerides (MCTs). Preferably, the lipid fraction comprises approximately 40% to about 60% by calories MCTs. MCTs are more easily absorbed and metabolized as compared to long chain triglycerides (LCTs). The use of MCTs will reduce the risk of the potential for nutrient malabsorption. A low omega-6 content and a high omega-3 content are provided. Preferably, the ratio of omega-6 to **omega-3 fatty acids** is less than 2.0:1. The low omega-6:omega-3 ratio reduces the incidence and severity of **inflammatory** reactions. **Omega-3 fatty acids** may modulate the negative, immune-mediated reactions brought about by high omega-6 intake. Therefore, oil blends which contain omega-3 (or are, at a minimum, low in omega-6) are preferred.

Accordingly, in an embodiment, a fish oil rich in **omega-3 fatty acids** is preferred, as fish oils contain two longer chain length **omega-3 fatty acids**: eicosapentaenoic acid (EPA, C22:5, n3) and docosahexaenoic acid (DHA, C22:6, n3). Soy oil is also preferred, in that it contains approximately 7% **linolenic acid** (C18:3, n3), in order to insure that a safe minimum level of shorter length **omega-3 fatty acids** is delivered, and also contains approximately 50-55% linoleic acid (C18:2, n-6), in order to insure that a safe minimum level of **omega-6 fatty acids** is delivered (essential fatty acids). In an embodiment of the present invention, the lipid component comprises by weight 50% MCT, 25% fish oil and 25% soy oil (includes soy oil and soy lecithin).

In addition to the ability of omega-3 to modulate **inflammatory** reactions, likewise, the antioxidant vitamins and minerals also reduce the incidence of severity of **inflammatory** reactions.

By utilizing a formulation having high protein and fat content, protein and energy requirements are met. However, at the same time, pursuant to the present invention, the formulation includes reduced water and carbohydrate content. This reduces the risk of over hydration, hyperglycemia, and carbohydrate intolerance.

Preferably, the formulation is approximately 35% to about 40%, by calories, carbohydrates. By way of example, the carbohydrates can be chosen from maltodextrin, corn starch, sucrose, and corn syrup solids.

In an embodiment, the present invention includes soluble or insoluble fiber, and/or carob pod powder or extract that is rich in insoluble tannin. In an enteral product, especially one to be provided by tube feeding, this provides anti-diarrhea characteristics. Magnesium can be reduced below U.S. RDA levels (400 mg/day), further limiting the potential for tube-fed induced diarrhea. An example of the use of tannin to reduce the incidence of diarrhea is set forth in U.S. patent application Ser. No. 887,360 entitled: "ENTERAL FORMULATION DESIGNED TO REDUCE DIARRHEA IN TUBE-FED PATIENTS" now abandoned, the disclosure of which is hereby incorporated herein by reference.

Preferably, anti-oxidant vitamins and minerals are increased to above the U.S. RDAs. This will insure that the patient receives at least 100% of the U.S. RDA as well as insure that any additional micronutrients that are necessary due to the patient's state will be provided. The formulation, in an embodiment, will provide approximately 5-6 mg/1500

Kcal of beta-carotene. Beta-carotene is a precursor for Vitamin A and has some unique antioxidant properties.

Of course, it will be appreciated that a variety of formulations are possible in accordance with the present invention. An example of a formulation in accordance with the present invention includes a formulation having a caloric density of 1.5 Kcal/ml. This is equivalent to 375 Kcal/250 ml which will, in a preferred embodiment, by one unit (can or container) of product.

In this embodiment, preferably, protein comprises 25%, by calories, of the product. This is equivalent to 94 grams/liter. A variety of different components are possible for the protein portion of the product. In an embodiment, casein hydrolysate plus arginine can be utilized. In a further embodiment, casein hydrolysate plus arginine plus proline can be utilized for the protein component. In a preferred embodiment, casein hydrolysate, whey hydrolysate and arginine can be utilized as the protein source.

In this preferred embodiment, the lipid component preferably comprises approximately 39% of the calories of the product. This will be equal to approximately 65 grams/liter. In the embodiment, approximately 50% of the lipid component is MCTs and 25% of the lipid component is fish oil. Preferably, 19 to 21% of the lipid component is soy oil and 4-6% soy lecithin. This will provide an omega-6:omega-3 ratio of approximately 1.8:1.

Preferably, in this embodiment, the carbohydrates comprise 36% of the calories. This is equivalent to 135 g/l. In the embodiment, maltodextrin and corn starch are used. Preferably, the maltodextrin provides approximately 84% of the carbohydrate source, where as, the corn starch provides approximately 14% of the carbohydrate source.

The total calories/nitrogen in this embodiment is approximately 90:1. The total non-protein calories/grams of nitrogen is approximately 67:1. Due to the use of the peptide-base protein source, osmolality will be approximately 600 mOsm/kgH.sub.2 O. It is envisioned that the shelf-life of the product will be approximately 12 months.

Pursuant to the present invention, the **omega-3 fatty acids** as a percent of the total calories of the product will be greater than 2.3%. Anti-**inflammatory** activity is believed to be achieved at 2.2% to 3% of the calories of the product. Anti-thrombotic and hypolipidemic is also believed to be a benefit of such high levels of omega-3. As set forth above, preferably, fish oil and soy oil are utilized. A number of potential beneficial effects are achieved by using fish oil.

Most typical nutritional products have less than 2.3% of the total calories as **omega-3 fatty acids.** To this end, the following commercially available products have the following **omega-3 fatty acid** content (as a % of total calories): IMPACT.RTM. 1.6%; IMMUN-AID.RTM. 1.0%; PEPTAMEN.RTM. VHP 1%; Promote 0.9%; TRAUMACAL.RTM. 0.3%; and PEPTAMEN.RTM. 0.2%.

In an embodiment of the present invention, the formulation of the present invention includes at least 3% of the total calories as arginine. Enhanced wound healing with arginine is believed to be provided at quantities greater than 3% of the total calories.

Additionally, in an embodiment, the present invention includes

significant amounts of proline. In an embodiment, the proline content is at least 2.0% of the total calories. Proline content as a percent of specific proteins is as follows: gelatin=16.1%; casein=9.6%; whey=5.7%; and soy=5.4%.

Still further, in an embodiment, ,the present invention can include significant amounts of cysteine. In an embodiment, the present invention can provide approximately 0.06% of the total calories as cysteine. This is substantially in line with ad-libitum diets. However, the present invention, in a preferred embodiment, provides 0.25% of the total calories as cysteine. This percentage of cysteine content can be achieved if approximately 40% of the hydrolysate is from whey protein hydrolysate. Preferably, a sufficient amount of cysteine is included in the composition to replenish intracellular glutathione in the patient. Cysteine content of various proteins is as follows: casein=0.3%; total milk products=0.9%; soy protein=1.2%; whey protein=2.0%; and egg white protein=2.5%.

Pursuant to the present invention, non-protein calories/grams of nitrogen (NPC/gN) is determined so as to provide a composition that spares the use of proteins as the calorie source. Patients with severe metabolic stress (trauma, burns) preferably should receive a product with an NPC/gN of less than 100:1 because of their increased protein requirements. Pursuant to the present invention, the formulation provides compositions having less than or equal to 70:1. The weight/nitrogen weight of certain proteins is as follows: arginine 3.11:1; glutamine 5.21:1; casein 6.25:1; protein 6.25:1; whey 6.38:1; proline 8.21:1; branched chain amino acids 8.79:1.

By way of example, and not limitation, examples of formulations of the present invention will now be given.

DETD FORMULA EXAMPLE NO. 1

A liquid, ready-to-use enteral product With protein at 25% of total calories: 87% from partially hydrolyzed casein and 13% from the free amino acid arginine. Carbohydrates would be 35-40% of calories. Lipids comprise 38-42% of calories, preferably a blend of medium chain triglycerides (50%), fish oil (25%), soy oil and soy lecithin (25% total of both soys). Vitamin and mineral content would meet preferably daily requirements in 1500 calories.

FORMULA EXAMPLE NO. 2

A liquid, ready-to-use enteral product with protein at 25% of total calories: 50% from partially hydrolyzed casein, 34% from partially hydrolyzed whey protein, 12% from the free amino acid arginine and 4% from the free amino acid proline. Carbohydrates would be 35-40% of calories. Lipids comprise 38-42% of calories, preferably a blend of medium chain triglycerides (50%), fish oil (25%), soy oil and soy lecithin (25% total of both soys). Vitamin and mineral content Would meet preferably daily requirements in 1500 calories.

FORMULA EXAMPLE NO. 3

A liquid ready-to-use enteral product made pursuant to the present invention may have the following nutrient profile:

NUTRIENT COMPOSITION	Per 250 ML

Protein	23.45	g
Carbohydrate	33.75	g
Fat	16.9	g
Vitamin A	1500	I.U.
Beta-Carotene	1.35	mg
Vitamin D	100	I.U.
Vitamin E	25	I.U.
Vitamin K	18.75	mcg
Vitamin C	250	mg
Thiamine (B.sub.1)	0.75	mg
Riboflavin (B.sub.2)	0.6	mg
Niacin	7	mg
Vitamin B.sub.6	1.0	mg
Folic Acid	135	mcg
Pantoth. Acid	3.5	mg
Vitamin B.sub.12	2.0	mcg
Biotin	100	mcg
Choline	112.5	mg
Taurine	37.5	mg
L-Carnitine	37.5	mg
Calcium	250	mg
Phosphorus	250	mg
Magnesium	100	mg
Zinc	9	mg
Iron	4.5	mg
Copper	0.75	mg
Manganese	1.0	mg
Iodine	40	mcg
Sodium	292	mg
Potassium	468	mg
Chloride	435	mg
Chromium	35	mcg
Molybdenum	55	mcg
Selenium	25	mcg

In this example, the protein source preferably comprises approximately 52% casein hydrolysate, approximately 35% whey hydrolysate, and approximately 13% L-arginine. The carbohydrate source preferably includes approximately 86% maltodextrin and approximately 14% corn starch. Lastly, the lipid source preferably includes approximately 50% from medium-chain triglycerides, approximately 25% from fish oil, approximately 19% from soy oil, and approximately 6% from soy lecithin.

By way of example, and not limitation, a contemplative example of the use of the present invention will now be given.

CONTEMPLATIVE EXAMPLE NO. 1

Two hundred patients admitted to intensive care units with moderate to severe trauma are nutritionally supported by the use of tube-fed enteral formulas. Half receive a whole protein based product at 1.0 calories/mL, with protein as 22% of calories (a combination of whole protein and free amino acid arginine), carbohydrates at 50-55% of calories and lipids at 20-25% of calories, with 25% as MCT and the remainder fish oil and sunflower oil. Vitamin and mineral U.S. RDAs met in 1500 calories (1500 mL). Half receive a formula described in this invention: a liquid, ready-to-use enteral product at 1.3-1.5 Kcal/mL with protein at 25% of total calories (87% from partially hydrolyzed casein and 13% from the free amino acid arginine), carbohydrates at 35-40% calories and lipids at 38-42% of calories, with half of the lipid as MCT, 25% fish oil and 25% soy oil and soy lecithin. Vitamin and mineral U.S. RDAs met in 1500

calories (1000 mL).

Many of the patients receiving the whole protein diet were unable to receive the recommended calorie and protein intakes of 2200-2500 calories and 140 grams protein because of intolerance and diarrhea and conflicts with the need to not overhydrate. By comparison, the elemental and calorically dense product described in this invention, it is believed, will be able to deliver 2250 calories and 140 grams protein in 1.5 liters/day with a minimal incidence of intolerance or diarrhea. When using APACHE scoring to predict outcomes, the patients fed the enteral diet described in this invention will, it is believed, have a shorter average length of stay and fewer **inflammatory** complications than would have been expected based on experiences with whole protein-based diets which contain less than 2.3% of calories as a mixture of **omega-3 fatty acids** (linolenic, EPA and DHA).

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

CLM What is claimed is:

1. A method for providing nutritional support to a trauma, burn or post-surgery patient comprising the step of enterally administering to the patient a therapeutically effective amount of a composition comprising: 22% to about 28% of the total calories as a protein source including protein hydrolysate and free amino acids, the protein hydrolysate including less than 35% by weight, peptides having a chain length of more than five amino acids; a lipid source; and a carbohydrate source.

2. The method of claim 1 wherein the free amino acids comprise less than 20% by weight of the protein source.

3. The method of claim 1 wherein the protein source comprises less than 20% by weight peptides having a chain length of more than nine amino acids.

4. The method of claim 1 wherein the composition includes a cysteine content of at least 0.25% of the total calories of the composition.

5. The method of claim 1 wherein the protein hydrolysate comprises 70% to 50% of casein hydrolysate and 30% to 50% whey hydrolysate.

6. The method of claim 1 wherein the lipid source includes a source of medium chain triglycerides, a source of **omega-3 fatty acids** and a source of **omega-6 fatty acids**.

7. The method of claim 1 wherein the protein hydrolysate is produced through use of pancreatic enzymes.

8. The method of claim 1 wherein the protein source contains substantially no whole proteins.

9. A method for providing nutritional support to a trauma, burn or post-surgery patient comprising the step of enterally administering to

the patient a therapeutically effective amount of a composition comprising: a protein source comprising approximately 80% to 85% by weight of protein hydrolysate and 15% to 20% of free amino acids; a lipid source; a carbohydrate source; and the composition having a caloric density of at least 1.3 Kcal/ml.

10. The method of claim 9 wherein the protein hydrolysate includes less than 35% by weight peptides having a chain length of more than five amino acids.

11. The method of claim 9 wherein the free amino acids comprise less than 20% by weight of the protein source.

12. The method of claim 9 wherein the protein source comprises less than 20% by weight peptides having a chain length of more than nine amino acids.

13. The method of claim 9 wherein the protein hydrolysate comprises 70% to 50% of casein hydrolysate and 30% to 50% of whey hydrolysate.

14. The method of claim 9 wherein the protein hydrolysate is produced through use of pancreatic enzymes.

15. The method of claim 9 wherein the protein source contains substantially no whole proteins.

16. A method for providing nutritional support to a trauma, burn or post-surgery patient comprising the step of enterally administering to the patient a therapeutically effective amount of a composition comprising: 22% to about 28% of the total calories as a protein source including protein hydrolysate and free amino acids, the protein hydrolysate comprising less than 20%, by weight, peptides having a chain length of more than nine amino acids; a lipid source; and a carbohydrate source.

17. The method of claim 16 wherein the protein hydrolysate comprises less than 35% by weight peptides having a chain length of more than five amino acids.

18. The method of claim 16 wherein the free amino acids comprise less than 20% by weight of the protein source.

19. The method of claim 16 wherein the protein hydrolysate is produced through use of pancreatic enzymes.

20. The method of claim 16 wherein the protein source contains substantially no whole proteins.

21. A method for providing nutritional support to a trauma, burn or post-surgery patient comprising the step of enterally administering to the patient a therapeutically effective amount of a composition comprising: a protein source including protein hydrolysate and free amino acids, the protein source having a cysteine content of at least 0.25% of the total calories of the composition; a lipid source; and a carbohydrate source.

22. A method for providing nutritional support to a trauma, burn or post-surgery patient comprising the step of enterally administering to the patient a therapeutically effective amount of a composition comprising: a protein source which provides about 22% to about 28% of the total calories and which includes protein hydrolysate and free amino

acids, the protein hydrolysate comprising less than 35%, by weight, peptides having a chain length of more than five amino acids and less than 20%, by weight, peptides having a chain length of more than nine amino acids; a lipid source which provides about 33% to 45% of the total calories, the lipid source including a source of medium chain triglycerides, a source of **omega-3 fatty acids** and a source of **omega-6 fatty acids**; and a carbohydrate source which provides 35% to about 40% of the total calories.

INCL INCLM: 514/021.000
INCLS: 514/002.000; 514/023.000; 514/054.000; 514/558.000; 514/560.000; 514/943.000; 426/072.000; 426/607.000; 426/656.000; 426/658.000; 424/DIG.013
NCL NCLM: 514/021.000
NCLS: 424/DIG.013; 426/072.000; 426/607.000; 426/656.000; 426/658.000; 514/002.000; 514/023.000; 514/054.000; 514/558.000; 514/560.000; 514/943.000
IC [6]
ICM: A61K038-00
ICS: A23J001-00; A23G003-00
EXF 514/21; 514/2; 514/23; 514/54; 514/558; 514/560; 514/943; 426/72; 426/607; 426/656; 426/658; 424/DIG.13
ARTU 181
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 8 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 1998133854 EMBASE
TI **.omega.-3 Fatty acid**-based lipid infusion in patients with chronic plaque psoriasis: Results of a double-blind, randomized, placebo-controlled, multicenter trial.
AU Mayser P.; Mrowietz U.; Arenberger P.; Bartak P.; Buchvald J.; Christophers E.; Jablonska S.; Salmhofer W.; Schill W.-B.; Kramer H.-J.; Schlotzer E.; Mayer K.; Seeger W.; Grimminger F.
CS Dr. P. Mayser, Department of Dermatology, Justus Liebig University Giessen, Gaffkystr. 14, D-35385 Giessen, Germany
SO Journal of the American Academy of Dermatology, (1998) 38/4 (539-547).
Refs: 28
ISSN: 0190-9622 CODEN: JAADDB
CY United States
DT Journal; Article
FS 013 Dermatology and Venereology
037 Drug Literature Index
LA English
SL English
AB Background: Profound changes in the metabolism of eicosanoids with increased concentrations of free arachidonic acid (AA) and its proinflammatory metabolites have been observed in psoriatic lesions. Free eicosapentaenoic acid (EPA) may compete with liberated AA and result in an **antiinflammatory** effect. Objective: Our purpose was to assess the efficacy and safety of intravenously administered fish-oil-derived lipid emulsion on chronic plaque-type psoriasis. Methods: A double-blind, randomized, parallel group study was performed in eight European centers. Eighty-three patients hospitalized for chronic plaque-type psoriasis with a severity score of at least 15 according to the Psoriasis Area and Severity Index (PASI) participated in a 14-day trial. They were randomly allocated to receive daily infusions with either a **.omega.-3 fatty acid**-based lipid emulsion (Omegavenous; 200 ml/day with 4.2 gm of both EPA and docosahexaenoic acid (DHA): 43 patients) or a conventional .omega.-6-lipid emulsion (Lipovenous: EPA+DHA < 0.1 gm/100 ml; 40 patients). The groups were well matched with respect to demographic data and psoriasis-specific medical history. Efficacy of therapy was evaluated by changes in PASI, in an overall assessment of psoriasis by the investigator, and a self-assessment

by the patient. In one center neutrophil 4- versus 5-series leukotriene (LT) generation and platelet 2- versus 3- thromboxane generation were investigated and plasma-free fatty acids were determined. Results: The total PASI score decreased by 11.2 .+-. 9.8 in the .omega.-3 group and by 7.5 .+-. 8.8 in the .omega.-0 group (p = 0.048). In addition, the .omega.-3 group was superior to the .omega.-6 group with respect to change in severity of psoriasis per body area, change in overall erythema, overall scaling and overall infiltration, as well as change in overall assessment by the investigator and self-assessment by the patient. Response (defined as decrease in total PASt of at least 50% between admission and last value) was seen in 16 of 43 patients (37%) receiving the .omega.-3 emulsion and 9 of 40 patients (23%) receiving **.omega .-6 fatty acid**-based lipid emulsion. No serious side effects were observed. Within the first few days of .omega.-3 lipid administration, but not in the .omega.-6 supplemented patients, a manifold increase in plasma-free EPA concentration, neutrophil leukotriene B5 and platelet thromboxane B3 generation occurred. Conclusion: Intravenous **.omega.-3- fatty acid** administration is effective in the treatment of chronic plaque- type psoriasis. This effect may be related to changes in **inflammatory** cicosanoid generation.

CT Medical Descriptors:
*psoriasis: DT, drug therapy
chronic disease
lipid composition
drug efficacy
drug screening
drug response
treatment outcome
human
male
female
major clinical study
clinical trial
randomized controlled trial
double blind procedure
multicenter study
controlled study
aged
adult
article
priority journal

Drug Descriptors:
*lipid emulsion: CT, clinical trial
*lipid emulsion: CB, drug combination
*lipid emulsion: DT, drug therapy
***omega 3 fatty acid: CT, clinical trial**
***omega 3 fatty acid: CB, drug combination**
***omega 3 fatty acid: DT, drug therapy**
***omega 6 fatty acid: CT, clinical trial**
***omega 6 fatty acid: CB, drug combination**
***omega 6 fatty acid: DT, drug therapy**
myristic acid: CT, clinical trial
myristic acid: CB, drug combination
myristic acid: DT, drug therapy
palmitic acid: CT, clinical trial
palmitic acid: CB, drug combination
palmitic acid: DT, drug therapy
palmitoleic acid: CT, clinical trial
palmitoleic acid: CB, drug combination
palmitoleic acid: DT, drug therapy
stearic acid: CT, clinical trial
stearic acid: CB, drug combination

stearic acid: DT, drug therapy
oleic acid: CT, clinical trial
oleic acid: CB, drug combination
oleic acid: DT, drug therapy
linoleic acid: CT, clinical trial
linoleic acid: CB, drug combination
linoleic acid: DT, drug therapy
linolenic acid: CT, clinical trial
linolenic acid: CB, drug combination
linolenic acid: DT, drug therapy
arachidonic acid: CT, clinical trial
arachidonic acid: CB, drug combination
arachidonic acid: DT, drug therapy
icosapentaenoic acid: CT, clinical trial
icosapentaenoic acid: CB, drug combination
icosapentaenoic acid: DT, drug therapy
docosahexaenoic acid: CT, clinical trial
docosahexaenoic acid: CB, drug combination
docosahexaenoic acid: DT, drug therapy
omegavenous
lipovenous
RN (myristic acid) 1715-79-3, 544-63-8; (palmitic acid) 57-10-3; (palmitoleic acid) 373-49-9; (stearic acid) 57-11-4, 646-29-7; (oleic acid) 112-80-1, 115-06-0; (linoleic acid) 1509-85-9, 2197-37-7, 60-33-3, 822-17-3; (**linolenic acid**) 1955-33-5, 463-40-1; (arachidonic acid) 506-32-1, 6610-25-9, 7771-44-0; (icosapentaenoic acid) 25378-27-2, 32839-30-8; (docosahexaenoic acid) 25167-62-8, 32839-18-2
CN Omegavenous; Lipovenous

L13 ANSWER 9 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 1998163284 EMBASE
TI Immunoregulatory and anti-**inflammatory** effects of n-3 polyunsaturated fatty acids.
AU Calder P.C.
CS P.C. Calder, Division of Human Nutrition, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton 5O16 7PX, United Kingdom
SO Brazilian Journal of Medical and Biological Research, (1998) 31/4 (467-490).
Refs: 172
ISSN: 0100-879X CODEN: RBPMB2
CY Brazil
DT Journal; General Review
FS 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LA English
SL English
AB 1. Fish oils are rich in the long-chain n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids. Linseed oil and green plant tissues are rich in the precursor fatty acid, .alpha.- **linolenic acid** (18:3n-3). Most vegetable oils are rich in the n-6 PUFA linoleic acid (18:2n-6), the precursor of arachidonic acid (20:4n-6). 2. Arachidonic acid-derived eicosanoids such as prostaglandin E2 are pro- **inflammatory** and regulate the functions of cells of the immune system. Consumption of fish oils leads to replacement of arachidonic acid in cell membranes by eicosapentaenoic acid. This changes the amount and alters the balance of eicosanoids produced. 3. Consumption of fish oils diminishes lymphocyte proliferation, T-cell-mediated cytotoxicity, natural killer cell activity, macrophage-mediated cytotoxicity, monocyte and neutrophil chemotaxis, major histocompatibility class II expression and antigen presentation, production of pro-**inflammatory** cytokines (interleukins 1 and 6, tumour necrosis factor) and adhesion molecule expression. 4. Feeding

laboratory animals fish oil reduces acute and chronic **inflammatory** responses, improves survival to endotoxin and in models of autoimmunity and prolongs the survival of grafted organs. 5. Feeding fish oil reduces cell-mediated immune responses. 6. Fish oil supplementation may be clinically useful in acute and chronic **inflammatory** conditions and following transplantation. 7. n-3 PUFAs may exert their effects by modulating signal transduction and/or gene expression within **inflammatory** and immune cells.

CT Medical Descriptors:
*immunoregulation
*fat intake
*diet supplementation
*cellular immunity
antiinflammatory activity
arachidonic acid metabolism
icosanoid metabolism
cell membrane
lymphocyte proliferation
cytotoxic t lymphocyte
neutrophil chemotaxis
antigen expression
antigen presentation
cytokine production
signal transduction
gene expression
graft survival
human
nonhuman
review
Drug Descriptors:
***omega 3 fatty acid**
fish oil
icosapentaenoic acid
docosahexaenoic acid
linseed oil
linolenic acid
omega 6 fatty acid
linoleic acid
arachidonic acid
prostaglandin e2
vegetable oil
major histocompatibility antigen class 2: EC, endogenous compound
interleukin 1: EC, endogenous compound
interleukin 6: EC, endogenous compound
tumor necrosis factor: EC, endogenous compound
cytokine: EC, endogenous compound
cell adhesion molecule

RN (fish oil) 8016-13-5; (icosapentaenoic acid) 25378-27-2, 32839-30-8; (docosahexaenoic acid) 25167-62-8, 32839-18-2; (linseed oil) 8001-26-1; (**linolenic acid**) 1955-33-5, 463-40-1; (linoleic acid) 1509-85-9, 2197-37-7, 60-33-3, 822-17-3; (arachidonic acid) 506-32-1, 6610-25-9, 7771-44-0; (prostaglandin e2) 363-24-6

L13 ANSWER 10 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 2
AN 1998126908 EMBASE
TI Dietary .alpha.-**linolenic acid** increases TNF-.alpha., and decreases IL-6, IL-10 in response to LPS: Effects of sesamin on the .DELTA.-5 desaturation of .omega.6 and **.omega.3** **fatty acids** in mice.
AU Chavali S.R.; Zhong W.W.; Forse R.A.
CS R.A. Forse, Department of Surgery, Beth Israel Deaconess Medical Center, 110 Francis Street, Boston, MA 02215, United States
SO Prostaglandins Leukotrienes and Essential Fatty Acids, (1998) 58/3 (185-191).
Refs: 41
ISSN: 0952-3278 CODEN: PLEAEU

CY United Kingdom
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
037 Drug Literature Index
LA English
SL English
AB Sesamin (a non-fat portion of sesame seed oil) inhibits .DELTA.-5 desaturase activity resulting in an accumulation of dihomo-.gamma.-**linolenic acid** (DGLA) which can displace arachidonic acid (AA) and decrease the formation of pro-**inflammatory** mediators. We investigated the effects of consumption of diets containing 0.25wt% sesamin and 15 wt% safflower oil (SO) (providing 12% of the added fat as linoleic acid) or a 15 wt% 2:1 mixture of linseed oil and SO (LOSO) (providing 6% .alpha.-**linolenic acid** and 6% linoleic acid) for 3 weeks on the liver membrane fatty acid composition and on the production of prostaglandin (PG) E2, TNF-.alpha., IL-6 and IL-10 in mice. Consumption of sesam-supplemented SO and LOSO diets resulted in a significant increase in the levels of 20:3.omega.6 (DGLA), suggesting that sesamin inhibited .DELTA.-5 desaturation of **.omega.6 fatty acids**. In animals fed LOSO diets, the levels of .alpha.-**linolenic acid**, eicosapentaenoic acid (EPA) and of docosahexaenoic acid (DHA) were elevated with a concomitant decrease of arachidonic acid (AA) in the liver membrane phospholipids. Further, in animals fed LOSO diets with or without sesamin, an increase in the circulating levels of TNF-.alpha. was associated with a concomitant decrease in PGE2. Despite a lack of differences in the levels of AA, the PGE2 levels were significantly lower in mice fed sesamin-supplemented SO compared to those fed SO alone. Thus, these data suggest that irrespective of the availability of a specific fatty acid as a substrate, through regulating the PGE2 synthesis, the production of TNF-.alpha. could be modulated.
CT Medical Descriptors:
*diet supplementation
*enzyme inhibition
*fatty acid desaturation
*lipid composition
***inflammation: ET, etiology**
liver membrane
nonhuman
female
mouse
animal experiment
controlled study
oral drug administration
article
priority journal
Drug Descriptors:
*interleukin 6: EC, endogenous compound
*interleukin 10: EC, endogenous compound
*tumor necrosis factor alpha: EC, endogenous compound
*sesamin: PD, pharmacology
*linoleic acid: PD, pharmacology
***omega 3 fatty acid: EC, endogenous compound**
***omega 6 fatty acid: EC, endogenous compound**
*arachidonic acid: EC, endogenous compound
*prostaglandin e2: EC, endogenous compound
lipopolysaccharide
fatty acid: EC, endogenous compound
safflower oil: PD, pharmacology
linseed oil: PD, pharmacology
polyunsaturated fatty acid: PD, pharmacology

RN (sesamin) 607-80-7, 7076-24-6; (linoleic acid) 1509-85-9, 2197-37-7, 60-33-3, 822-17-3; (arachidonic acid) 506-32-1, 6610-25-9, 7771-44-0; (prostaglandin e2) 363-24-6; (safflower oil) 8001-23-8; (linseed oil) 8001-26-1

L13 ANSWER 11 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 1998056401 EMBASE
TI Impact of dietary fat on Th1/Th2 cytokine gene expression in the pancreas and gut of diabetes-prone BB rats.
AU Kleemann R.; Scott F.W.; Worz-Pagenstert U.; Ratnayake W.M.N.; Kolb H.
CS Dr. H. Kolb, Diabetes Research Institute, Auf'm Hennekamp 65, D-40225 Dusseldorf, Germany
SO Journal of Autoimmunity, (1998) 11/1 (97-103).
Refs: 39
ISSN: 0896-8411 CODEN: JOAUEP
CY United Kingdom
DT Journal; Article
FS 003 Endocrinology
022 Human Genetics
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
048 Gastroenterology
LA English
SL English
AB The effect of dietary n-3 or n-6 polyunsaturated fatty acids on the development of autoimmune insulitis was analysed in diabetes-prone BB rats. Litter-matched groups of rats received a standard open formula NIH-07 (National Institutes of Health, NIH) diet enriched with 10% fish oil, 10% **flaxseed oil** or with 10% palm oil plus 2% cholesterol during the period of insulitis onset (50-70 days of age). Analysis of cytokine gene expression in pancreatic RNA revealed an increase of IFN-.gamma. and a decrease of IL-10 mRNA with onset of insulitis. When compared to unsupplemented NIH, none of the three fat-enriched diets depressed the rise of IFN-.gamma. gene expression or the influx of leukocytes into islets. However, all of the fat-enriched diets led to significantly higher IL-10 mRNA levels. Although a specific anti-**inflammatory** effect of fish oil was not seen in the pancreas, a clear shift of the Th1/Th2 cytokine mRNA ratio towards Th2 was seen in the gut-associated immune system. We conclude that diets high in fat support IL-10 without suppressing IFN-.gamma. gene expression in islet **inflammation**. A special anti-**inflammatory** effect of fish oil was not seen in pancreatic lesions of BB rats, although there was strong modulation of the IFN-.gamma./IL-10 mRNA ratio in the gut associated immune system.
CT Medical Descriptors:
*fat intake
*insulitis
*helper cell
*diabetes mellitus
immunogenetics
pancreas
intestine
autoimmunity
nonhuman
rat
animal experiment
animal model
animal tissue
article
priority journal
Drug Descriptors:
*cytokine: EC, endogenous compound
omega 3 fatty acid
omega 6 fatty acid

fish oil
linseed oil
palm oil
cholesterol
gamma interferon: EC, endogenous compound
interleukin 10: EC, endogenous compound
messenger rna: EC, endogenous compound
polyunsaturated fatty acid
RN (fish oil) 8016-13-5; (linseed oil) 8001-26-1; (palm oil) 8002-75-3; (cholesterol) 57-88-5; (gamma interferon) 82115-62-6

L13 ANSWER 12 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 1998067614 EMBASE
TI Positive role of immune nutrition on metabolism in sepsis and multi-organ failure.
AU Georgieff M.; Tugtekin I.F.
CS Dr. M. Georgieff, Universitatsklinik fur Anasthesiol., Steinhovelstr. 9, 89070 Ulm, Germany
SO Kidney International, Supplement, (1998) 53/64 (S80-S83).
Refs: 43
ISSN: 0098-6577 CODEN: KISUDF
CY United States
DT Journal; Conference Article
FS 006 Internal Medicine
024 Anesthesiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB Critically ill patients with systemic **inflammatory** response syndrome (SIRS) and multi-organ failure are at great risk of nosocomial infections due to a reduced immune status. There is growing evidence from in vitro studies and animal models that the reduced immune response might be improved by the so-called immunomodulatory nutrition. Based on these studies there are now some commercially available enteral or parenteral solutions with immunomodulatory substrates, such as n-3 polyunsatured fatty acids (PUFAs), arginine and nucleotides. Recently, enteral nutrition with this experimental formula reduced the hospital length of stay and the frequency of acquired infections in critically ill patients. The increasing knowledge about the metabolic effects of these nutritions offers therapeutic potential for the future, and might reduce the mortality of critically ill patients from nosocomial infections. However, at present, studies are necessary to find the best time for beginning and duration of the feeding. In addition, the optimal dosage and composition of these pharmacologically active substances has to be investigated.
CT Medical Descriptors:
*sepsis: TH, therapy
*multiple organ failure: TH, therapy
*immunomodulation
*enteric feeding
*parenteral nutrition
critical illness
high risk patient
hospital infection: CO, complication
hospital infection: PC, prevention
immune status
human
nonhuman
clinical trial
conference paper
priority journal
Drug Descriptors:
***omega 3 fatty acid**

*arginine
*nucleotide
*immunomodulating agent
omega 6 fatty acid
linoleic acid
linolenic acid
arachidonic acid
icosapentaenoic acid
docosahexaenoic acid
medium chain triacylglycerol
short chain fatty acid
glutamine

RN (arginine) 1119-34-2, 15595-35-4, 7004-12-8, 74-79-3; (linoleic acid) 1509-85-9, 2197-37-7, 60-33-3, 822-17-3; (**linolenic acid**) 1955-33-5, 463-40-1; (arachidonic acid) 506-32-1, 6610-25-9, 7771-44-0; (icosapentaenoic acid) 25378-27-2, 32839-30-8; (docosahexaenoic acid) 25167-62-8, 32839-18-2; (glutamine) 56-85-9, 6899-04-3

L13 ANSWER 13 OF 33 USPATFULL
AN 97:91511 USPATFULL
TI Enternal formulations for treatment of **inflammation** and infection
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PA Beth Israel Deaconess Medical Center, Inc., Boston, MA, United States (U.S. corporation)
PI US 5674853 19971007 <--
AI US 1995-399542 19950307 (8)
RLI Continuation of Ser. No. US 1994-228599, filed on 15 Apr 1994, now patented, Pat. No. US 5397778 which is a continuation-in-part of Ser. No. US 1994-201682, filed on 25 Feb 1994, now abandoned
DT Utility
FS Granted
REP

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EXNAM Primary Examiner: Leary, Louise

LREP Lahive & Cockfield, LLP
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN No Drawings
AB The present invention features saponin containing enteral formulations for treatment of infection and **inflammation**. These saponin containing formulations are particularly useful in conjunction with oils rich in .omega.3 polyunsaturated fatty acids such as fish oils and flax oil but also show benefits with .omega.6 rich oils such as borage oil, black currant seed oil, canola oil and rapeseed oil. These formulations may also contain a lignan from the sesamin family.

PARN REFERENCE TO RELATED APPLICATIONS

This application is a Continuation of application Ser. No. 08/228,599 filed on Apr. 15, 1994 now U.S. Pat. No. 5,397,778 which is a continuation-in-part of U.S. patent application Ser. No. 08/201,682, entitled "Anti-**inflammatory** and Infection Protective Effects of Sesamin-Based Lignans", filed Feb. 25, 1994, now abandoned, on an application of the presents inventors, the disclosure of which is incorporated herein by reference.

SUMM BACKGROUND OF THE INVENTION

The present invention relates to dietary manipulation for the treatment of disease. More particularly, the present invention relates to the use saponins in an enteral formulation for treatment of infection and **inflammation.**

The last decade has seen an explosion in the exploration of the interaction between diet and disease. In particular, the effects of various amino acids and lipids in the diet on a variety of conditions including heart disease, hypercatabolic states, liver disease, immunosupresssion, and infection treatment have been uncovered. Often, the effects are far removed from the norm and as such are unexpected. One of the most important developments of this type has been the discovery that by changing the dietary lipid content, positive effects in health treatment beyond plasma fat modification could be achieved. While the early work in modifying lipid content and type in diet came from an understanding that saturated fats cause particular problems in heart disease, later work determined that not just the use of polyunsaturated fats but also the type of polyunsaturated fat was important.

There are three major families of polyunsaturated fatty acids: .omega.3, .omega.6 and .omega.9. The names are based on location of the closest double bonds to the methyl end of the fatty acid; that is, if the closest double bond is between the third and fourth carbon atoms from the methyl group, the molecule is classified as an **.omega.3 fatty acid** while if the double bond is between the 6th and 7th carbon atoms, it is classified as an **.omega.6 fatty acid**. Mammals can desaturate or elongate fatty acid chains but cannot interconvert fatty acids from one family to another. The most important dietary fatty acids are the C.sub.18 and C.sub.20 fatty acids, primarily linoleic (C18:2.omega.6), **linolenic acid** (C18:3.omega.3), .gamma.-**linolenic acid** (C18:3.omega.6) and dihomo-.gamma.-**linolenic acid** (C20:3.omega.6). Manipulation of the content of these fatty acids changes the ratio of arachidonic, eicosapentanoic, and decahexanoic acids (C20:4.omega.6, C20:5.omega.3, and C22:6.omega. receptively) and can cause far reaching effects in terms of immunosuppression, response to hypercatabolic states, and infection. For example, U.S. Pat. No. 4,752,618, issued Jun.

21, 1988 on an application of Mascioli et al., the disclosure of which is incorporated herein by reference, discloses the beneficial effects of .**omega.3 fatty acids** in the treatment of infection. In U.S. Pat. No. 5,260,336, issued Nov. 3, 1993 on an application of Forse et al., the disclosure of which is also incorporated herein by reference, concerns a method of minimizing the effect of catabolic illness or infection using an oil such as olive oil which is rich in .omega.9 fatty acids. Other similar patents and articles, such as U.S. Pat. No. 4,810,726, issued Mar. 7, 1989 on an application of Bistrian et al., the disclosure of which is also incorporated herein by reference, disclose other means of treating illness using fatty acid dietary manipulation.

The "culprit" in many diets appears to be the high level of .**omega.6 fatty acids**, primarily linoleic acid, a precursor for the formation of arachidonic acid which is a substrate for the production of pro **inflammatory** dienoic eicosanoids including PGE.sub.2 and TxA.sub.2 which can lead to elevated levels of thromboxane A.sub.2 and related prostanoids. Elevation of these prostanoids has been linked to problems in response to endotoxin challenge and other infection states. Accordingly, the new wave in diets has been to minimize the .**omega.6 fatty acid** content (which, although an essential fatty acid, is not needed in the quantities found in most commercial oils) while maximizing the .**omega.3 fatty acids** (e.g., fish oil) and .omega.9 fatty acids (e.g., olive oil). Similarly, although sesame oil has long been promoted as having medicinal benefits, it is only recently that the effects have been traced to sesamin (and its related lignans) in the sesame oil. In fact, U.S. patent application Ser. No. 08/201,682, filed Feb. 25, 1994, on an application of the same inventors, discloses that sesamin can promote resistance to infection and reduce **inflammation**. Thus, materials which modify lipid content in the diet may have important and surprising health effects.

The present invention uses saponins to treat infection and reduce **inflammation**. It has also been found that these saponins can work in concert with other agents such as fish oils to provide quicker (and consequently better) protection against infection.

Saponins are surface active triterpene or sterol glycosides. Although the saponins are found mainly in plants, they have also been found in certain marine animals such as echinoderms like starfish and sea cucumbers. Most saponins are non-toxic when taken orally, but many are toxic upon i.m. or i.v. injection. Saponins are most often ingested by man in legumes such as chick peas and soy beans. In fact, it has been theorized that legumes rich in saponins may reduce the threat of heart disease based, in part, on the finding that saponins can reduce plasma cholesterol levels in animals. See, e.g., Newman et al., Poultry Science 37 42-45(1957).

However, the main medicinal use for saponins appears to be their properties as immunostimulating substances or adjuvants. Reports of immunopotentiating advantages using saponins go back over fifty years (see, e.g., Thibault and Richou, C. R. Soc. Biol. 121 718-721 (1936)). While saponins are available from many sources, much of the work on immunostimulation has used saponins derived from the inner bark of the South American soaptree, Quillaja saponaria Molina. These saponins, normally designated as the Quill A saponins, remain the principal medicinal saponins in use today.

Although many other medicinal uses have been hypothesized for saponins, there has been no systematic proof that any effects other than use as an adjuvant is medicinally feasible. However, saponins have been found in some plants used in traditional or folk remedies. For example, saponins are present in ginseng which has long been used in Asia for treatment of a variety of conditions. Similarly, other homeopathic remedies also may contain saponins. The recent interest in homeopathic remedies has lead to a further exploration of the properties of materials such as saponins.

Accordingly, an object of the invention is to provide an enteral dietary supplement containing saponins.

Another object of the invention is to provide a means of treating infection and/or **inflammation** using saponins.

A further object of the invention is to provide a dietary supplement useful in improving the effects of .**omega**.3 **fatty acids** on treatment of infection.

An additional object of the invention is to provide a dietary supplement useful in improving the uptake of polyunsaturated fatty acids (e.g., EPA and DHA) in tissue.

A still further object of the invention is to provide a method of treating infection and/or **inflammation** using dietary manipulation.

These and other objects and features of the invention will be apparent from the following description and the claims.

SUMMARY OF THE INVENTION

The present invention features enteral formulations for treatment of **inflammation** and infections, as well as methods of treatment itself. These formulations are based on the surprising properties of saponins, a material that is often used as an adjuvant but not as the medicament itself. The saponins are effective with standard enteral formulations such as safflower oil dietary supplements and appear to have additive, or even synergistic, effects with .**omega**.3 **fatty acid** formulations such as those derived from fish oil or linseed oil. The saponins can also be used with sesamin and related lignans from sesame oil to provide particularly advantageous diets. These saponins could also be included in other food products such as margarines and butter as well as dietary supplements. Such other food products and dietary supplements are included in the enteral formulations herein.

More particularly, the present invention features an enteral formulation adapted for treatment of infection or **inflammation** in a patient which includes an effective amount of a saponin as an active ingredient. The term "effective amount" means a sufficient amount of the saponin to cause the clinical effect in terms of anti-**inflammation** and/or anti-infection properties. This effective amount can vary due to a number of factors including type of saponin and personal metabolism. For Quill A, one of the most readily available

saponins, this effective amount appears to be about 0.1%-1.0% by weight of the enteral diet, with a 0.25% amount being preferred. For other saponins, with different purification and potency, different effective amounts may easily be determined.

The enteral formulation useful in the invention may include particular fatty acids or other materials which have similar anti-**inflammatory** properties. For example, the previously cited U.S. Pat. No. 4,752,618 discloses that **.omega.3 fatty acids** may have anti-infection properties. An enteral formulation which includes these **.omega.3 fatty acids** in conjunction with the saponins is, therefore, advantageous. Preferred sources of .omega.3 are the fish oils, and linseed (flax) oil, most preferably the oils derived from cold water fish which have at least 10% of their lipid content in **.omega.3 fatty acids** and flax oil which contains approximately 55% **linolenic acid** (18:3 .omega.3). Examples of the useful cold water fish include menhaden and sardine. In fact, as is shown later in the examples, the addition of saponins to an enteral formulation containing **.omega.3 fatty acids** causes less lagtime until the beneficial effects of the **.omega.3 fatty acids** occur and increased uptake of **.omega.3 fatty acids** into tissue. These saponins may also yield beneficial effects with other dietary oils such as borage oil, black currant seed oil, canola oil, and rapeseed oil.

Another additive useful in an enteral formulation is a lignan of the sesamin family. Previously cited U.S. patent application Ser. No. 08/201,682, filed Feb. 24, 1994, discloses the anti-infection and anti-**inflammatory** properties of these lignans. The lignans preferred include sesamin, episesamin, sesaminol, espisemsaminol, and sesamolin. A combination therapy including these lignans and the saponins may be particularly advantageous.

Any enteral formulation preferably includes essential amino acids, essential fatty acids, and/or essential vitamins and minerals. The enteral formulations of the present invention may be in the form of a dietary supplement or used as a total enteral feeding regimen. If the later, these essential nutrients are required while even in a supplement, the addition insures that the patient is obtaining these nutrients.

The enteral formulation such as is previously described are particularly useful in treating infection and **inflammation**. In fact, these formulations may be used in at risk patients to prevent possible infection or **inflammation**. Further, when used with the other formulations such as the **.omega.3 fatty acids**, the time to effective action may be reduced.

The following description and non-limiting examples further elucidate the invention.

DETD DETAILED DESCRIPTION

The present invention provides an enteral formulation useful in treating **inflammation** and/or infection. This enteral formulation includes an effective amount of a saponin such as Quill A, possible in conjunction with a diet rich in **.omega.3**

fatty acids or a diet containing a lignan such as sesamin. As such, saponins show remarkable promise as additives in treating infection states, particularly acute infections e.g., sepsis.

The following examples, which all use saponins in enteral diets, further explain the invention.

Example 1

This example explains the procedure used to create the diets used for test purposes. The two diets basic diets were made, a safflower oil diet (SO) which had large quantities of **.omega.6 fatty acids**, primarily in the form of linoleic acid, and a fish oil (FO) diet which had a large percentage of **.omega .3 fatty acids**. The oil portion of the safflower oil diet was made by taking 52 g of safflower oil (SVO Specialty Products, Culberton, Mont.) and mixing it with 88 g of palm oil and 10 g of Trisum, a high oleic sunflower oil. The fish oil diet used menhaden oil, which has 32% .omega.3 polyunsaturated fats, primarily in the form of eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), as the fish oil. The fish oil portion of the fish oil diet was made by blending 8 g safflower oil, 125 g of fish oil, 35 g of palm oil and 10 g of Trisum. These physical mixtures of oils were prepared to maintain the saturated, monounsaturated, and polyunsaturated fat contents identical in both experimental diets. However, the polyunsaturated fatty acids in the former is .omega.6 type and in the latter is .omega.3. One hundred fifty grams of each oil mixture was added to 850 g of AIN-76, a fat-free basal diet which contained essential minerals and vitamins. For each 1000 g of either enteral diet, 15% by weight was in form of fat with the fat calories being approximately 30% of the total (as recommended by the Surgeon General).

The combination of the fat and the AIN-76 fat-free basal diet had 0.05% t-butyl hydroxy tolulene added as an antioxidant, and the diets were stored in individual daily rations, flushed with nitrogen to minimize oxidation, at 4.degree. C. The animals were fed ad libium every day before dusk.

Separate groups of Balb/c mice were maintained on the safflower oil diet, the fish oil diet, and the two diets supplemented with saponins. Plasma was sampled at 4, 7 and 10 days and the fatty acid compositions of phospholipids in the plasma were determined by gas chromatography following a thin layer of chromatography.

The relative mole percent of individual fatty acids (including linoleic acid and arachidonic acid) incorporated into the plasma phospholipids and the tissues were determined. There was substantially no difference in the fatty acid pattern for the safflower oil diet vs. the safflower oil with saponin diet but the fish oil diet vs. fish oil with saponin diet was another matter. At day 4, the relative percentages of eicosapentanoid acid and decahexenoic acid (DHA) were twice as high in the plasma phospholipids of mice consuming the fish oil with saponins diet as compared with the fish oil alone. By day 7, the differences disappeared. However, the levels of tissue polyunsaturated **.omega.3 fatty acids** increased at day 7 and remained elevated until day 10.

Example 2

In this example, Balb/C mice were maintained ad libium on one of the diets described in Example 1, the safflower oil diet, for three weeks. Safflower oil diets are commonly used for enteral nutrition. A first

group received just the safflower oil diet (SO) while the second group had the safflower oil diet supplemented with 0.25% saponins (SO+). There were twenty animals in the first group and seventeen in the second group.

At the end of three weeks, all the animals in both groups underwent cecal ligation and puncture. To perform this procedure, the mice were anaesthetized and then shaved over the anterior abdominal wall. A midline incision, approximately 2 cm long, was made, sufficient to expose the cecum and adjacent intestine. With a 3-0 silk suture, the cecum was tightly ligated at its base without causing bowel obstruction.

The cecum was then punctured twice with a 22 gauge needle, gently squeezed to exude feces and to insure that the two puncture holes did not close. The abdominal incision was then closed and 1 ml of saline was administered subcutaneously for fluid resuscitation. This cecal ligation and puncture is a widely accepted form of infection model to resemble abdominal sepsis. See, e.g., C. Baker et al., "Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model," Surgery (August 1983), pp. 331-335. Survival of the mice is the normal measure of treatment effectiveness.

In addition, ten animals were fed each diet to serve as controls and were a sham operated; this means, that the abdominal operation was performed but cecal ligation and puncture was not carried out.

TABLE 1

Diets	24 hours	48 hours	72 hours	96 hours
SAFFLOWER OIL (SO)	20 (100)	14 (70)	6 (30)*	4 (20)*
SAFFLOWER OIL + SAPONINS (SO+)	17 (100)	16 (94)	15 (88)**	15 (88)**

Table 1 shows the survival on the SO diet vs. the SO+ diet. While all the animals in each group were alive at 24 hours, the number of animals alive at 48, 72 and 96 hours decreases rapidly for the safflower oil group while the group being treated with the safflower plus saponin diet shows very little mortality. The first number is the number of animals remaining alive while the second is a percent remaining alive. At 72 hours, the number of animals surviving is statistically significant ($p<0.05$ using a student t test) while at 96 hours, the data are even better ($p<0.01$). The groups of animals consuming the diets supplemented with saponins showed no mortality.

Accordingly, this shows that adding the saponins to a safflower oil diet has significant anti-infection effects.

Example 3

The beneficial effects of feeding diets enriched with safflower oil (15 wt %=30% total calories) supplemented with or without saponins (0.25%) was tested in an infection model. Groups of 10 female Balb/c mice, 6-8 weeks old, were fed the two diets for 3 weeks. The plasma levels of

thrombxane B.sub.2 (TBX.sub.2), tumor necrosis factor (TNF)-.alpha. and other proinflammatory mediators were determined in plasma 90 minutes after an interperitonial injection of lipopolysacchride (LPS) (20 mg/kg).

TABLE 2

	SAFFLOWER OIL (SO)	SO + SAPONINS
TXB.sub.2 (pg/ml*)	466 .+-. 98	257 .+-. 48.sup.#
TNF-.alpha. (pg/ml*)	380 + 100	100 .+-. 40.sup.#

*means .+-. S.D of determinations following LPS i.p injection in mice (n 10 in each group.)
#p < 0.05.

The increase in survival of animals in Example 2 were associated with significantly lower concentrations (45%) of the LPS-induced TBX.sub.2 and TNF-.alpha. in the circulation while the AA content, a precursor for the formation of dienoic eicosanoids (such as TBX.sub.2), was unchanged for the groups of mice fed safflower oil diets containing saponins as described in Example 1. These data suggest that saponins possess anti-**inflammatory** properties which may include inhibiting the activities of phospholipase A.sub.2 or cyclooxygenase enzymes. Further, the ability of saponins to markedly lower (74%) the LPS-induced in vivo production of TNF-.alpha. suggests a possible mechanism by which dietary saponins confer protection against infection irrespective of the type of polyunsaturated fatty acid in the diet. These data indicate that inclusion of saponins in an enteral formulation containing different types of polyunsaturated fatty acids (.omega.3, .omega.6, or .omega.9) could benefit critically ill patients.

Example 4

In this experiment, Balb/c mice were again maintained on either the safflower oil diet alone or the safflower oil diet supplemented with 0.25% of the saponin Quill A. Spleens were isolated aseptically at 1, 2 and 3 weeks and single cell suspensions were prepared. One million spleen cells were stimulated with either concanavalin A (Con A-1 mg/ml) or lyopopolysacchride (lps-10 .mu.g/ml) for twenty-four hours, both of which are known to induce the production of proinflammatory mediators. Cell free supernatants were collected and the amounts of prostaglandin E.sub.2 (PGE.sub.2) were determined by immunoassay. The PGE.sub.2 levels in the supernatants from the spleen cells of the animals treated with the saponins were significantly lower (see Table 2) than those with the safflower oil diet alone on day 7 ($p<0.05$). After two or three weeks of feeding, the mean concentrations of the PGE.sub.2 were not significantly different. These data suggest the saponins exhibited anti-**inflammatory** properties and that feeding safflower oil diets with saponins may have selected a cell population which participated in defending the host against infection.

TABLE 3

	Con A	LPS
SAFFLOWER OIL	114 .+-. 20	248 .+-. 32

(SO)
SAFFLOWER OIL + 70 .+-. 13
153 .+-. 11
SAPONINS (SO+)

All valves in pg/ml at day 7.

Since it is known that fish oil diets will provide anti-infection properties, the ability of the saponin addition to provide a more rapid incorporation of **.omega.3 fatty acids** in the fatty acid profiles of the phospholipids in the plasma and in the tissues suggest that this may speed the action of the fish oil. If so, this effect may be important in treating infection, particularly with post-operative patients.

The foregoing examples are merely exemplary and one skilled in the art may determine other enteral diets and methods of treatment using such an enteral diet which falls within the scope of the present invention. The invention is defined not by these examples but rather by the following claims.

CLM What is claimed is:

1. An enteral formulation for the treatment of infection or **inflammation** in a patient comprising a source of dietary polyunsaturated fatty acids as a significant part of the fat content of said enteral formulation and a saponin as an active ingredient in an amount effective to reduce the level of infection or **inflammation** in said patient.

2. The enteral formulation of claim 1 wherein said source of dietary polyunsaturated fatty acids is selected from the group consisting of fish oils and vegetable oils rich in **.omega.3 fatty acids.**

3. The enteral formulation of claim 2 wherein said source of **.omega.3 fatty acids** is a fish oil having at least 10% of the lipid content as **.omega.3 fatty acids.**

4. The enteral formulation of claim 1 further comprising a lignan selected from the group consisting of sesamin, episesamin, sesaminol, episesaminol, and sesamolin.

5. The enteral formulation of claim 4 wherein said lignan is added to said enteral formulation in the form of sesame oil.

6. The enteral formulation of claim 4 wherein said lignan is added to said enteral formulation in the form of purified lignan.

7. The enteral formulation of claim 1 further comprising essential amino acids.

8. The enteral formulation of claim 1 further comprising essential vitamins and minerals.

9. The enteral formulation of claim 1 wherein said enteral formulation includes a dietary oil selected from the group consisting of borage oil, black currant seed oil, canola oil and rapeseed oil.

10. A method of treating infection in patients comprising the step of enteral administration of an effective amount of an enteral formulation to treat said infection in said patients, said enteral formulation comprising a source of dietary polyunsaturated fatty acids as a

significant part of the fat content of said enteral formulation and a saponin as an active ingredient in an amount effective to reduce the level of infection or **inflammation** in said patients.

11. The method of claim 10 wherein said source of dietary polyunsaturated fatty acids is selected from the group consisting of fish oils and vegetable oils rich in .**omega.3 fatty acids**.

12. The method of claim 11 wherein said source of .**omega.3 fatty acids** is a fish oil having at least 10% of its lipid content as .**omega.3 fatty acids**.

13. The method of claim 10, wherein said enteral formulation further comprises a lignan selected from the group consisting of sesamin, episesamin, sesaminol, episesaminol and sesamolin.

14. The method of claim 13 wherein said lignan is added to said enteral formulation in the form of sesame oil.

15. The method of claim 13 wherein said lignan is added to said enteral formulation in the form of a purified lignan.

16. The method of claim 10, wherein said enteral formulation further comprises an oil selected from the group consisting of borage oil, black currant seed oil, canola oil and rapeseed oil.

INCL INCLM: 514/025.000
INCLS: 514/464.000; 514/468.000; 514/783.000; 514/825.000; 514/886.000; 514/887.000; 514/904.000; 514/905.000; 424/195.100; 424/DIG.013
NCL NCLM: 514/025.000
NCLS: 424/755.000; 424/764.000; 424/765.000; 424/776.000; 424/DIG.013; 514/464.000; 514/468.000; 514/783.000; 514/825.000; 514/886.000; 514/887.000; 514/904.000; 514/905.000
IC [6]
ICM: A01N043-04
ICS: A01N043-30; A01N025-00; A01N065-00
EXF 514/25; 514/464; 514/468; 514/783; 514/825; 514/886; 514/887; 514/904; 514/905; 424/195.1; 424/DIG.13
ARTU 121
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 14 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 3
AN 1998026617 EMBASE
TI [Polyunsatured fatty acids and diabetes].
ACIDES GRAS POLYINSATURES ET DIABETE.
AU Raccah D.; Coste T.; Gerbi A.; Vague P.
CS D. Raccah, Service de Nutrition, Maladies Metaboliques, Endocrinol., Hopital de la Timone, F 13385 Marseille Cedex 5, France
SO Cahiers de Nutrition et de Dietetique, (1997) 32/6 (349-358).
Refs: 55
ISSN: 0007-9960 CODEN: CNDQA8
CY France
DT Journal; Article
FS 003 Endocrinology
037 Drug Literature Index
LA French
SL English; French
AB The two essential fatty acids, linoleic and alpha **linolenic acids**, are polyunsaturated fatty acids, representative of two families: n-6 and n-3, which participate in the composition of cell membrane phospholipids, and influence membrane-bound protein activity, like enzymes. The two essential fatty acids have a similar metabolism, and

are the precursors of eicosanoids including prostaglandins which exert a role in vasomotricity, and leucotrienes implicated in **inflammation** . In diabetes, there is a decrease of delta 6 desaturase activity. This abnormality leads to an impairment of active metabolite production, such as prostaglandins, eicosapentaenoic and docosahexaenoic acids. In parallel, an impairment of phospholipid fatty acid composition is observed, accompanied by a decrease of enzyme activity, particularly the Na/K ATPase. These disorders are involved in the physiopathology of diabetic complications such as neuropathy and cardiomyopathy. Therapeutic perspectives deal with omega 3 polyunsaturated fatty acids supplementation in diabetic cardiomyopathy and myocardial ischemia, gamma **linolenic acid** supplementation and vasoactive prostaglandin analogs in diabetic neuropathy.

CT Medical Descriptors:
*diabetes mellitus
*cardiomyopathy: CO, complication
*cardiomyopathy: DT, drug therapy
*diabetic neuropathy: CO, complication
*diabetic neuropathy: DT, drug therapy
fatty acid metabolism
icosanoid metabolism
prostaglandin metabolism
lipid composition
enzyme activity
diet supplementation
human
nonhuman
article
Drug Descriptors:
*linoleic acid: DT, drug therapy
*linoleic acid: EC, endogenous compound
***linolenic acid: DT, drug therapy**
***linolenic acid: EC, endogenous compound**
***gamma linolenic acid: DT, drug therapy**
***gamma linolenic acid: EC, endogenous compound**
polyunsaturated fatty acid: DT, drug therapy
polyunsaturated fatty acid: EC, endogenous compound
omega 6 fatty acid: DT, drug therapy
omega 6 fatty acid: EC, endogenous compound
omega 3 fatty acid: DT, drug therapy
omega 3 fatty acid: EC, endogenous compound
membrane phospholipid: EC, endogenous compound
icosanoid: EC, endogenous compound
prostaglandin: EC, endogenous compound
adenosine triphosphatase (potassium sodium): EC, endogenous compound

RN (linoleic acid) 1509-85-9, 2197-37-7, 60-33-3, 822-17-3; (**linolenic acid**) 1955-33-5, 463-40-1; (gamma **linolenic acid**) 1686-12-0

L13 ANSWER 15 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 97019707 EMBASE
DN 1997019707
TI Modulation of human hepatocyte acute phase protein production in vitro by n-3 and n-6 polyunsaturated fatty acids.
AU Wigmore S.J.; Fearon K.C.H.; Ross J.A.
CS Dr. J.A. Ross, University Department of Surgery, Royal Infirmary of Edinburgh, Lauriston Place, Edinburgh EH3 9YW, United Kingdom
SO Annals of Surgery, (1997) 225/1 (103-111).
Refs: 36
ISSN: 0003-4932 CODEN: ANSUA5
CY United States
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LA English

SL English

AB Objective: The authors investigate the role of a variety of essential polyunsaturated fatty acids on the spontaneous and interleukin-6 stimulated production of acute phase proteins by isolated human hepatocytes. Summary Background Data: The altered production of acute phase proteins by the liver is one of the principal effects of the systemic **inflammatory** response in human disease. It has been shown that polyunsaturated fatty acids have certain anti-**inflammatory** properties that potentially are mediated through altered prostaglandin or proinflammatory cytokine production. However, the effect of polyunsaturated fatty acids on the responsiveness of the human hepatocyte to proinflammatory cytokines has not been studied in detail. Methods: Hepatocytes isolated from human livers were maintained in primary culture in the presence of a variety of bovine serum albumin-complexed fatty acids. The influence of these fatty acids on hepatocyte acute phase protein production was assessed, in the presence and absence of recombinant interleukin-6, by measurement of acute phase proteins by enzyme-linked immunosorbent assay. Results: Eicosapentaenoic and gammalinolenic acid increased spontaneous production of .alpha. 1-antichymotrypsin and prealbumin but decreased spontaneous production of transferrin and haptoglobin from isolated human hepatocytes. Eicosapentaenoic and gammalinolenic significantly increased interleukin-6 stimulated production of C-reactive protein and .alpha. 1-antichymotrypsin but reversed the stimulatory effect of interleukin-6 on haptoglobin production. These fatty acids also reversed the inhibitory effect of interleukin-6 on prealbumin production. Conclusions: These results show that certain fatty acids have the potential to modulate spontaneous and cytokine-induced alterations in human hepatic acute phase protein metabolism. These data indicate the presence of complex mechanisms of regulation of human hepatic protein metabolism by fatty acids, and further study will be required to establish the nature of their influence in vivo.

CT Medical Descriptors:
*acute phase response
*liver cell
*protein metabolism
article
cell count
cell function
cell isolation
cell stimulation
controlled study
enzyme linked immunosorbent assay
human
human cell
liver cell culture
metabolic regulation
priority journal
Drug Descriptors:
*acute phase protein: EC, endogenous compound
***omega 3 fatty acid**
***omega 6 fatty acid**
*recombinant interleukin 6
alpha 1 antichymotrypsin: EC, endogenous compound
bovine serum albumin
c reactive protein: EC, endogenous compound
gamma linolenic acid
haptoglobin: EC, endogenous compound
icosapentaenoic acid
prealbumin: EC, endogenous compound
transferrin: EC, endogenous compound

RN (c reactive protein) 9007-41-4; (gamma **linolenic acid**) 1686-12-0; (haptoglobin) 9087-69-8; (icosapentaenoic acid) 25378-27-2,

32839-30-8; (transferrin) 82030-93-1

L13 ANSWER 16 OF 33 USPATFULL
AN 96:120918 USPATFULL
TI Use of an emulsion to prepare an intravensously administered medicament for treating skin diseases
IN Schlotzer, Ewald, Oberursel, Germany, Federal Republic of
Mayser, Peter, Biebertal, Germany, Federal Republic of
Grimminger, Friedrich, Butzbach, Germany, Federal Republic of
Seeger, Werner, Lahnau, Germany, Federal Republic of
Weidler, Burghard, Rosbach, Germany, Federal Republic of
Sommermeyer, Klaus, Rosbach, Germany, Federal Republic of
Thomas, Martin, Frankfurt am Main, Germany, Federal Republic of
PA Fresenius AG, Germany, Federal Republic of (non-U.S. corporation)
PI US 5589508 19961231 <--
WO 9306812 19930415 <--
AI US 1994-211644 19940420 (8)
WO 1992-EP2285 19921002
19940420 PCT 371 date
19940420 PCT 102(e) date
PRAI DE 1991-4133694 19911011
DT Utility
FS Granted
REP US 4678808 Jul 1987 514/560.000 Ward et al.
US 5352700 Oct 1994 514/560.000 Frithz et al.
US 5434183 Jul 1995 514/549.000 Larsson-Backstr om
DE 3722540 Jun 1990
WO 9008544 Aug 1990
REN Burczyk, A., "Bedeutung unges attigter Fetts auren in Nahrung und Kosmetik," Seifen- Ole-Fette-Wachse 115:462-463 (1989). (Summary Only).
Chapkin, R. S. et al., "Dietary Influences of Evening Primrose and Fish Oil on the Skin of Essential Fatty Acid-Deficient Guinea Pigs," J. Nutrition 117:1360-1370 (Aug. 1987).
Horrobin, D. F., "Low Prevalances of Coronary Heart Disease (CHD), Psoriasis, Asthma and Rheumatoid Arthritis in Eskimos: Are They Caused by . . . ?," Medical Hypotheses 22:421-428 (Apr. 1987).
Lowe, N. J. et al., "Linoleic Acid Effects on Epidermal DNA Synthesis and Cutaneous Prostaglandin Levels in Essential Fatty Acid Deficiency," J. Invest. Dermatol. 70:200-203 (Apr. 1978).
Martinez, M. and Ballabriga, A., "Effects of Parenteral Nutrition with High Doses of Linoleate on the Developing Human Liver and Brain," Lipids
22:133-138 (1987).
Simopoulos, A. P., "Summary of the NATO Advanced Research Workshop on Dietary .omega.3 and .omega.6 Fatty Acids: Biological Effects and Nutritional Essentially," J. Nutrition 119:521-528 (Apr. 1989).
Ziboh, V. A. et al., "Effects of Dietary Supplementation of Fish Oil on Neutrophil and Epidermal Fatty Acids", Arch. Dermatol. 122:1277-1282 (Nov. 1986).
EXNAM Primary Examiner: Geist, Gary; Assistant Examiner: Frazier, Barbara S.
LREP Sterne, Kessler, Goldstein & Fox P.L.L.C.
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 5 Drawing Page(s)
AB An emulsion containing one or several polyunsaturated, long-chain omega-3 and/or **omega-6 fatty acids** or their pharmaceutically tolerable esters or salts, as well as usual adjuvants and additives, is used to produce an intravenously administered medicament for treating skin diseases, in particular **inflammatory** skin diseases, as well as diseases of the dermatitis or eczema group. Preferably, fatty acids containing 18-22 carbon atoms, as well as their pharmaceutically tolerable esters of salts, are used. These acids or their pharmaceutically tolerable esters or salts may be used in their pure form or as components of oils, such as fish oil, highly-purified fish oil concentrates, linseed oil, primrose oil, borage oil or soya oil. Specially preferred are the

pharmaceutically tolerable esters or salts of said acids, in particular the pharmaceutically tolerable esters, especially those derived from eicosapentaenic acid. The emulsions may be further intravenously administered in the framework of a combined therapy with presently known therapies of skin diseases.

SUMM This application is a 371 of PCT/EP92/02285, filed Oct. 2, 1992.

TECHNICAL FIELD

The invention concerns the use of an emulsion that contains one or more, polyunsaturated, long-chain **omega-3 fatty acids** and/or **omega-6 fatty acids** or their pharmaceutically tolerable esters or salts for, respectively, the intravenous administration for the treatment of skin diseases, or to prepare an intravenously administered medicament for treating skin diseases, particularly **inflammatory** skin diseases, as well as diseases of the dermatitis and eczema family, in particular of skin diseases of the dermatitis and eczema family.

Today, skin diseases represent a high percentage of diseases in humans and animals with, for example, psoriasis are among the most common skin diseases, from which approximately 1 to 2% of the population suffers.

STATE OF THE ART

The necessity of essential fatty acids for the structure and function of the skin is known. Using animal models, rats have who were given a diet free of these fatty acids have exhibited developmental impairments and skin alterations with reddening, dandruff, and hyperkeratoses in the region of the sebaceous glands. Other alterations included increased effluvium, hyperproliferation with increased epidermal cell turn-over, impaired healing of wounds, and increased transepidermal water loss. These skin alterations were reversible upon substitution. The principal sources of the essential fatty acids, which are classified by the position of their first double bond as either omega-3 or **omega -6 fatty acids**, are, primarily, cold-water fish (for **omega-3 fatty acids**) or vegetable oils (for **omega-6 fatty acids**). In mammals, the presence of different enzymes which give unsaturation and enzymes which give elongation can lead to the formation of additional secondary products.

The observation that populations that exhibit a high level of **omega-3 fatty acid** consumption (for example, Eskimos) exhibit only one-twentieth the incidence of psoriasis of comparable populations who nourish themselves primarily with **omega-6 fatty acids**, has led to several clinical studies which examined the effect of a diet rich in fish oil, that is, an oral application of fish oil, on the course of various forms of psoriasis (for example, The Lancet, Feb. 20, 1988, page 378; Journal of the American Academy of Dermatology, 1988, volume 18, pages 1267 through 1273; British Journal of Dermatology, 1987, 117, pages 599-613).

While the indications are not uniform for chronic, constant, common psoriasis, they do, however, agree on the fact that a clinical improvement results from a linear dosage/effect relationship. For the exudative forms (exanthematous psoriasis, pustular psoriasis), but also for psoriatic arthritis, the trend appears to be toward uniformly

positive indications, although the published number of cases is still small. This therapeutic reasoning is based on the suppression and antagonization of the metabolism of arachidonic acid vital to the pathogenesis by inclusion of the structurally-related eicosapentaenoic acid (EPA) in the lipid metabolism of both keratinocytes as well as neutrophile granulocytes. Granulocytes seem to play an important role, particularly in **inflammatory** forms of psoriasis, a fact that is supported by increased function parameters as well as the histological characteristics of the infiltration of the epidermis and the formation of so-called Munroe's micro-abscesses. In particular in pustular forms, the increased chemotactic and pro-**inflammatory** activity results in the formation of clinically visible pustules on skin altered by **inflammation**.

As a potent chemotactic substance, LTB4, a lipoxygenase product of the arachidonic acid that has been found in increased levels in psoriatic lesions, can explain these findings. In addition, it stimulates keratinocyte proliferation in cell cultures.

The oral ingestion of eicosapentaenoic acid (**omega-3 fatty acid**) contained in fish oil, the metabolism of the arachidonic acid (**omega-6 fatty acid**) can be competatively inhibited to such an extent that biologically less potent metabolites are produced: in the case of LTB4, for example, the significantly less chemotacticly effective LTB5. This is explained by the uptake of eicosapentaenoic acid in place of arachidonic acid in the cell membrane, and by the competition of this substance for the enzymes, cyclooxygenase and lipoxygenase. The studies carried out showed that the oral treatment of skin diseases with fish oil (for example, in the form of the oral administration of fish oil capsules) requires a long treatment period (up to several months), during the course of which in some cases very large amounts (for example, 10 to 75 g) of fish oil must be taken daily, whereby correspondingly severe gastrointestinal complaints (for example, nausea, a feeling of fullness, retching, eruction) arise during the course of the treatment. A further aspect of this type of oral fish oil therapy is the poor patient compliance.

Other forms of treatment of skin diseases, including severe psoriasis, include the treatment with retinoids such as eretinate and acitretin. Significant disadvantages of this treatment are hyperlipidemia, including hypertriglyceridemia, hypercholesteremia and a reduced level of high-density lipoprotein cholesterol (HDL-C). For oral therapy lasting for a longer period of time, the side-effects induced by retinoid treatment represent a potent risk for severe cardiovascular illnesses. Other presently known treatment methods for skin diseases are treatment with cignolin, non-steroid antiphlogistics, antihistamines, or corticosteroids, as well as photo or balneophototherapy. But this treatment methods also lead to considerable side-effects, whereby burns occur as the most common side-effect for treatment with cignolin and for photo or balneophototherapy, while for treatment with corticosteroids, skin atrophy has been observed. What all these standard treatments, with the exception of corticosteroid therapy, have in common however, is the disadvantage that they require a relatively long treatment period. Corticosteroids act very rapidly, however, because they lead to skin atrophy, they can only be used for a short period. Treatment with non-steroid antiphlogistics or with antihistamines are, in addition, only alleviating measures, whose side-effects lead to stomach complaints

or induce fatigue.

DESCRIPTION OF THE INVENTION

The object of the invention is thus a suitable agent and process for the treatment of skin diseases, particularly **inflammatory** skin diseases, as well as diseases of the dermatitis and eczema family, in particular of skin diseases of the dermatitis and eczema family, that does not display the disadvantages of the known agents and processes, and with whose aid visible treatment successes can be achieved within a shorter treatment period.

In accordance with the invention, it was surprisingly found that, by the intravenous administration of fat emulsions containing one or more polyunsaturated, long-chain **omega-3 fatty acids** and/or **omega-6 fatty acids** or their pharmaceutically tolerable esters or salts for the treatment of skin diseases, particularly **inflammatory** skin diseases, as well as diseases of the dermatitis and eczema family, in particular of skin diseases of the dermatitis and eczema family, after only a few days of treatment a therapeutic success is already visible, and the disadvantages or side-effects associated to the known standard treatments can be avoided.

Aside from the rapid onset of the effect with intravenous treatment, other advantages over previous standard treatment methods lie in the improved patient compliance--that is, the more ready acceptance of the treatment on the part of the patients--reduced strain for the patients due to the intravenous treatment, no appearance of gastrointestinal problems, as well as a shorter hospital stay for inpatients, a shorter treatment period, and therefore a reduction in the treatment costs. While the oral treatment of the skin diseases described above requires several weeks (at least 6 weeks) up to several months with the daily administration of 20 or more fish oil capsules, the intravenous treatment in accordance with the invention with the emulsion requires only a few days of treatment with a daily infusion period of approximately one hour.

Preferred are emulsions for use in accordance with the invention with polyunsaturated, long-chain, omega-3 and/or **omega-6 fatty acids** containing 18 to 22 C atoms, as well as their esters and salts. Examples of suitable **omega-3 fatty acids** are .alpha.-**linolenic acid**, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DCHA), whereby preferably EPA and DCHA, particularly EPA, is used. One or more of the **omega-3 fatty acids** can be present in the emulsions. The acids or their pharmaceutically tolerable esters or salts can be used either in their pure form, or as a component of fish oil, highly purified fish oil concentrates or linseed oil, preferably as fish oil or highly purified fish oil concentrations. Suitable fish oils are, for example, those types which are technically recovered in substantial quantities from cold-water fish. Examples of such fish oils include pilchard oil, menhaden oil, Peruvian fish oil, sardine oil, salmon oil, herring oil, and mackerel oil. Preferred are highly purified fish oil concentrations such as are produced from mackerel, sardines, herrings, or salmon, Whereby these have an EPA content of 20 to 40%, preferably at least 26% (based on the fatty acid methyl ester of the fish oil concentrate). Examples of suitable fish oil emulsions are described in DE PS 37 22 540, to which the reader is referred.

Examples of suitable **omega-6 fatty**

acids include linoleic acid, .gamma.-**linolenic acid**, dihomo-.gamma.-**linolenic acid**, and arachidonic acid, whereby .gamma.-**linolenic acid** and dihomo-.gamma.-**linolenic acid** are preferred. The emulsion applied in accordance with the invention can contain one or more **omega-6 fatty acids**.

Omega-6 fatty acids or their pharmaceutically tolerable esters or salts can be used in either their pure form or in the form of components of oils, for example, primrose oil, borage oil, or soybean oil. Preferably primrose oil is used.

The pharmaceutically tolerable esters and salts of the cited omega-3 and/or **omega-6 fatty acids** are preferably used, whereby the pharmaceutically tolerable esters of these acids are particularly preferred. Pharmaceutically tolerable esters of the omega-3 and **omega-6 fatty acids** include the ethyl esters or glycerin esters, for example, mono-, di-, or triglycride esters, whereby triglycerides are preferred. Sodium salts are suitable as pharmaceutically tolerable salts.

The emulsions employed in accordance with the invention can contain either:

a) **Omega-3 fatty acids**, their pharmaceutically tolerable esters or salts in pure form or as a component of oils as were cited above, or;

b) **Omega-6 fatty acids**, their pharmaceutically tolerable esters or salts in pure form or as a component of oils as were cited above, or;

c) A mixture of the acids cited in a) and in b) above, or their pharmaceutically tolerable esters or salts.

For example, in the emulsions employed in accordance with the invention can contain a mixture of fish oil and other oils such as primrose oil, borage oil, or soybean oil, whereby the ratio of fish oil to the other oils (by weight) is most suitably in the range between 9:1 and 1:9. For example, the ratio of fish oil to primrose oil and/or borage oil can be 1:1, and the ratio of fish oil to soybean oil can be 7:1.

The **omega-3 fatty acids** and/or **omega-6 fatty acids** or their pharmaceutically tolerable ester or salts are present in quantities of 5 to 45% by weight, preferably in quantities of 10 to 30% by weight and, in particular, in quantities of 10-20% by weight in the emulsions employed in accordance with the invention.

Preferred in accordance with the invention are those emulsions based solely on **omega-3 fatty acids**, their esters or salts in pure form or in the form of components of oils, such as were cited above, in particular, those based on fish oils.

The emulsions employed in accordance with the invention also contain at least one, physiologically safe emulsifier. Suitable are phospholipids with an animal or vegetable origin, preferably those phospholipids which contain EPA as a polyunsaturated fatty acid. Ovolecithin is particularly suitable.

The emulsifier is present in the emulsion in quantities of 5 to 15% by weight (based on the fat content), preferably in quantities of 5 to 12% by weight (based on the fat content).

In addition, vitamin E, for example in the form of tocopherol or pharmaceutically safe tocopherol ester, for example, tocopherol acetate, can also be present in the emulsion in quantities of 0.15 to 1.5% by weight (based on the fat content), to act as an antioxidant.

As additional additives, the emulsion employed in accordance with the invention can also contain such as the common aids as conventional emulsion stabilizers, isotonic additives and/or co-emulsifiers as well as selenium compounds, if required. A suitable selenium compound is, for example, Na.sub.2 SeO.sub.3 .times.0.5H.sub.2 O.

Suitable isotonic additives include the commonly employed isotonic agents such as glycerin, glucose, xylose, and sorbite, whereby glycerin is preferred.

A suitable, preferable emulsion employed in accordance with the invention has, for example, the following composition:

Fish oil	100	mg/ml
Glycerin (isotonic agent)	25	mg/ml
Ovolechitin	12	mg/ml
Vitamin E	0.15	mg/ml
Water (for injection) to make 1 ml.		

The fish oil used in the above-cited composition is preferably highly refined fish oil that has been enriched in **omega-3 fatty acids** in triglyceride compounds by means of a specific procedure as is described in DE PS 37 22 540. It contains at least 40% by weight **omega-3 fatty acids**. The total EPA and DCHA content of the fish oil as triglyceride components lies in the range of 25 to 50% by weight, preferably in the range of 35 to 50% by weight (each value determined on the basis of the surface percentage in a gas chromatogram). In the fish oil, the EPA and DCHA can be present in varying quantitative ratios, that can be determined by measuring the respective surfaces in the gas chromatogram. The quantitative ratios depend on the nature of the fish oil used, and on the degree of enrichment of **omega-3 fatty acids** achieved. Fish oils in which EPA and DCHA as triglyceride components are present in a quantitative ratio of EPA to DCHA in the range between 0.5 to 2.6 (surface ratio in the gas chromatogram), are the fat emulsions whose use is preferred.

The fat emulsions employed in accordance with the invention are oil-in-water emulsions (O/W) for which the external, phase consists of distilled water, suitable for intravenous administration.

The emulsions employed in accordance with the invention are produced in the conventional manner. A suitable process is described, for example, in DE PS 37 22 540.

In accordance with the invention, the emulsions can be employed for the intravenous administration for the treatment of skin diseases such as:

1. Skin diseases that are induced or maintained by derivatives of arachidonic acid formed by granulocytes or their sub-populations (neutrophils, eosinophils), by keratinocytes or by both, whereby in particular, the following are to be mentioned:

a) **Inflammatory** skin diseases such as common psoriasis, pustular psoriasis, psoriatic arthritis, allergic vasculitis;

b) Diseases of the dermatitis and eczema family such as constitutional neurodermatitis, contact dermatitis (allergic/toxic);

c) **Inflammatory** skin diseases with eosinophilia (hypereosinophilia syndrome, eosinophilic cellulitis, hypereosinophilic dermatitis);

d) Vesiculated dermatoses such as common pemphigus, vesiculated pemphigoid;

e) Photodermatoses, i.e. acute photodermatitis, polymorphous photodermatoses.

2. Skin diseases in conjunction with impaired function of the immune system, in particular with over-stimulated immune function, whereby the following are particularly to be named: lupus erythematosus and other, so-called collagenoses, areal alopecia, graft versus host disease, pilaris.

The emulsions are intravenously employed in accordance with the invention, particularly in the treatment of **inflammatory** skin diseases as well as diseases of the dermatitis and eczema family, in particular for the treatment of diseases of the dermatitis and eczema family. In accordance with the invention, it was surprisingly discovered that, with intravenous administration of fat emulsions, aside from achieving higher effectiveness levels, acute anti-**inflammatory** effects can also be achieved. In accordance with the existing view, the effect of the **omega-3 fatty acids**, in particular the effect of the EPA, is based on an inclusion of these fatty acids in place of arachidonic acid in the cell membrane, from which they are released upon appropriate stimuli by phospholipases and, depending on the enzymatic composition of the cell in question, are transformed into corresponding mediators with cyclo- and/or lipoxygenases. However, with this concept, effects can only be achieved after a multiple-week therapy period, because eicosapentaenoic acid is only obtained over a complex, indirect path via a modulation of the cellular phospholipid composition. In accordance with the invention, it was surprisingly discovered that, with respect to the therapeutic employment in diseases effecting the cutaneous system, an intravenous application of a fish oil emulsion allows an acute therapeutic intervention in the previously cited diseases. This may possibly be the result of the fact that, in an **inflammatory** focus, free EPA is also absorbed directly by cells capable of eicosanoid synthesis in competition to free extracellular arachidonic acid, and can be metabolized by them. Therefore, the acute, anti-**inflammatory**, therapeutic intervention is a new possibility in the treatment of the diseases cited above.

With respect to the diseases listed, acute immunodulatory effects result via the alteration of the mediator profile (cyclo- and lipoxygenase products), as well as the composition of the cell membrane, because the fluidity and therefore the possibility for antigen presentation at the cell membrane changes with the composition of its lipid portion. Further, with the substitution of **omega-3 fatty acids**, the properties of lymphocytes (i.e. suppression of killer cells) and macrophages (reduced eicosapentaenoic acid production with unaltered phagocytic capability and production of oxygen radicals) are altered.

The intravenous administration of the emulsion in cases of skin diseases

in accordance with the invention can, in addition, take place within the framework of a combination therapy, in particular in combination with a therapy with:

a) retinoids, systemic;

b) cignolin, externally;

c) phototherapy (SUP, PUVA) or balncophototherapy;

d) corticosteroids (internal/external)

e) non-steroidal antiphlogistics, and/or;

f) antihistamines.

Thus, when retinoids are used in cases of psoriasis and other **inflammatory** skin diseases, the additional intravenous administration of **omega-3 fatty acids** leads, aside from the additional, anti-**inflammatory** effect, to the observation of a rapid drop in the retinoid-induced serum lipid increase. In the combination therapy with the intravenous administration of the emulsions and photo or cignolin therapy, the erythema threshold can be raised so that the dosage of conventional therapeutic agents can be more rapidly increased, or their undesirable side-effects can be lessened. In the combination therapy of intravenous administration of the emulsions and the conventional treatment with corticosteroids, the usually required dosage of corticosteroids is reduced and, consequently, the skin atrophy produced by them is lessened. The combination of the intravenous administration of the emulsions with non-steroidal antiphlogistics or antihistamines correspondingly permits the reduction of the conventionally required dosage and time period of administration of the agents, and therefore also a reduction of the disadvantages normally associated with their use.

For the intravenous administration in the treatment of skin diseases, an amount of fat emulsions that corresponds to 0.01 to 0.3 g, preferably 0.05 to 0.15 g of the cited fatty acid(s), their esters or salts, per kg of body weight per day, is suitable. Thus, for example, an amount of fat emulsion can be employed as corresponds to 0.01 to 0.2 g, preferably 0.05 to 0.1 g of EPA, its esters or salts, per kg of body weight per day, or which corresponds to 0.05 to 0.5 g of oil (for example, fish oil and/or primrose oil), per kg of body weight per day, preferably 0.1 to 0.5 g, in particular, 0.1 to 0.3 g of fish oil per kg of body weight per day, can be employed.

The fat emulsions in accordance with the invention are not toxic.

DRWD BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1a-1b show graphs depicting the mean score values (FIG. 1a) and relative scores (FIG. 1b) for erythema improvement from example 1 ("T") and example 2 ("K"), as listed under "1. Erythema" herein.

FIGS. 2a-2b show graphs depicting the mean score values (FIG. 2a) and relative scores (FIG. 2b) for scaling improvement from example 1 ("T") and example 2 ("K"), as listed under "2. Scaling" herein.

FIGS. 3a-3b show graphs depicting the mean score values (FIG. 3a) and

relative scores (FIG. 3b) for exudation improvement from example 1 ("T") and example 2 ("K"), as listed under "3. Exudation" herein.

FIGS. 4a-4b show graphs depicting the mean score values (FIG. 4a) and relative scores (FIG. 4b) for subjective improvement from example 1 ("T") and example 2 ("K"), as listed under "4. Subjective improvement" herein.

FIGS. 5a-5b show graphs depicting the mean score values (FIG. 5a) and relative scores (FIG. 5b) for improvement in itching from example 1 ("T") and example 2 ("K"), as listed under "5. Improvement in itching" herein.

DETD METHODS OF REALIZING THE INVENTION

The following examples serve to further clarify the invention presented here.

EXAMPLE 1

A fat emulsion suitable for intravenous administration was produced from the components listed below:

Fish oil	100	mg
Glycerin	25	mg
Ovolecithin	12	mg
Vitamin E	0.15	mg
Water (for injection) to make	1	ml.

The fish oil used is highly-refined and contains at least 40% by weight of **omega-3 fatty acids** and was produced in the manner described in example 1 of DE PS 37 22 540.

The fat emulsion was produced in the same manner as described in example 5 of DE PS 37 22 540.

The toxicological study of the 10% fish oil emulsion produced above, using two species--beagles and Charle's River rats--carried out over 4 weeks showed that these fish oil emulsions displayed no indications of any kind of systemic toxicity or intravascular irritation after intravenous administration of doses up to 5,000 mg/kg of body weight per day.

EXAMPLE 2

A 10% fat emulsion suitable for intravenous administration was produced from the components listed below:

Soybean oil	100	mg
Glycerin	25	mg
Ovolecithin	12	mg
Water (for injection) to make	1	ml.

The fat emulsions produced in example 1 and 2 were examined in the following manner with respect to their effectiveness when intravenously administrated for skin diseases:

Ten patients suffering from acute, exanthematous psoriasis were included in the study, whereby the clinical examination of the emulsion in accordance with example 1 was studied with six patients, while four patients received the emulsion in accordance with example 2. The individual patient data are summarized in table 1.

The study was carried out under double-blind conditions. Twice per day at intervals of 12 hours over a period of 10 days, the patients were intravenously given 50 ml of the respective fat emulsion (emulsion from example 1 or 2). The patients were examined daily with regard to the alteration in the clinical picture and, at certain intervals and using conventional methods, with regard to the changes in the EPA metabolites, the triglyceride content, the cholesterol content, and the IgE. The determination of the EPA metabolites was carried out with the aid of high-performance liquid chromatography (HPLC), using both the reverse as well as the straight-line method. Triglycerides and cholesterol were determined with the aid of enzymatic chromatometry (GPO-PAP method and CHOD-PAP method respectively). The following criteria were employed in the evaluation of the clinical picture:

Erythema, scaling, exudation, subjective improvement, and improvement in itching.

The individual results gathered in the study are as follows, expressed as mean score values:

Mean score values (A1-A10)

1. Erythema					
Day	1	2	3	4	5
T (6)	32.6	31.8	26	25.8	23.5
K (4)	29	28.5	26.75	26.5	24.5
Day	6	7	8	9	10
T (6)	22.5	21.3	19.8	17.3	16.8
K (4)	24	22.75	22	21.75	20.5

Relative score (%)		
Day	1	Day 10
T (6)	100	51.5
K (4)	100	70.7

2. Scaling					
Day	1	2	3	4	5
T (6)	29	27.3	24.5	23.5	20.3
K (4)	20.5	18.25	16.75	16.75	16.25
Day	6	7	8	9	10
T (6)	20.1	18	16.8	15.5	15.2
K (4)	16.25	15.5	15.25	15.25	15.25

Relative score (%)		
Day	1	Day 10
T (6)	100	52.4

K (4)	100			74.4	

3. Exudation

Day	1	2	3	4	5
T (6)	32.4	31.4	28.4	27.8	26
K (4)	9.25	9.75	9.75	9.75	9.75

Day	6	7	8	9	10
T (6)	25.2	22.4	21.4	18.8	17.6
K (4)	9.75	9.75	9.75	9.75	9.75

Relative score (%)

Day	1	Day 10
T (6)	100	54.3
K (4)	100	105

Note: No exudative component could be determined for one of the patients who was treated with the emulsion from example 1, as well as for two of the group who were treated with the emulsion from example 2.

T=Emulsion from example 1.

K=Emulsion from example 2.

4. Subjective improvement

Day	1	2	3	4	5
T (6)	18.8	18	22	23.5	23.3
K (4)	23.25	24.25	23	23.75	23.25

Day	6	7	8	9	10
T (6)	24.1	24.8	27.6	28.5	28.8
K (4)	23.5	24	22.25	23.75	23.75

Relative score (%)

Day	1	Day 10
T (6)	100	153
K (4)	100	102

5. Improvement in itching

Day	1	2	3	4	5
T (6)	3.5	3	4.5	5.5	5.1
K (4)	3.5	3.5	3.25	3.75	3.75

Day	6	7	8	9	10
T (6)	5.3	5.1	5.2	5	5.8
K (4)	3.75	3.75	4	3.75	4

Relative score (%)

Day	1	Day 10
T (6)	100	167.7
K (4)	100	114

Note:

1. For criteria 1-4, a maximum score value of 50 can be achieved; for criterion 5, a maximum value of 10.

2. Criterion 5 (improvement in itching) is part of criterion 4 (subjective improvement), that is, it is also included in this evaluation.

3. For criteria 1-3, a drop in the score indicates an improvement; for criteria 4 and 5, a deterioration. For clarification, the mean score values for the individual appearance picture of the skin and the subjective feelings respectively, are summarized in FIGS. 1 to 5. In addition, table 2 also shows the values for EPA metabolites, triglyceride content, cholesterol, and IgE determined during the course of the study.

The results achieved in the studies show that, for the intravenous administration of the emulsion in accordance with example 1, a noticeable improvement in the clinical condition occurred within a very few days, while, with the emulsion in accordance with example 2, a mild improvement in the clinical condition took place in all patients after a

few days. The improvement in the clinical condition correlates with the increase of the EPA metabolites and, in addition, with the absolute level of the serum IgE value, so that, in particular, a neurodermatitis can be influenced with the intravenous treatment in accordance with the invention. Aside from a mild venous irritation, no side-effects were observed.

EXAMPLE 3

Example 1 was repeated, with the exception that, in place of fish oil, the same amount of primrose oil was employed.

EXAMPLE 4

Example 1 was repeated, with the exception that 50 mg of the fish oil used in example 1 was replaced by 50 mg of primrose oil. In the intravenous administration of the fat emulsions obtained in accordance with this example, similar examination results were obtained to those described above for example 1.

EXAMPLE 5

Example 1 was repeated, with the exception that 1 .mu.g of selenium in the form of Na.sub.2 SeO.sub.3 .times.5H.sub.2 O was added to the emulsion produced in example 1. The intravenous administration for skin diseases of the emulsion produced in this manner achieved the same results as those described for example 1.

TABLE 1

No.	Patient	Age	Sex	Weight [kg]	Diagnosis	Spread [%]	Disease duration
A1	HG	27	Male	73	Acute ex. ps.	20	12 years
A2	EL	42	Male				

				95	Acute ex. ps.								
						25	20 years						
A3	MO	21	Male										
				67	Acute ex. ps.								
						18	10 years						
A4	ZH	47	Male										
				118	Acute ex. ps.								
						30	20 years						
A5	TF	30	Male										
				72	Acute ex. ps.								
						10	20 years						
A6	LJ	65	Male										
				66	Acute ex. ps.								
						10	0.5 years						
A7	HF	28	Male										
				71	Acute ex. ps.								
						12	15 years						
A8	HS	62	Male										
				95	Acute ex. ps.								
						90	31 years						
A9	HK	55	Male										
				88	Acute ex. ps.								
						35	36 years						
A10													
	GU	25	Female										
				65	Acute ex. ps.								
						15	2 years						

TABLE 2

No.	Medication	Score change (d1 = 100%) [%] E	S	EX	SB	J	EPA Metabolites	Triglycerides	Cholesterol	IgE
A1	T	-58								
		-56	-76							
				+45	+50	+786%				
							-46%	-3%		780
A2	K	-17								
		-30	-11							
				+50	+300					
						-26%	+50%	+31%		161
A3	K	-50								
		-100								
			--	+14	+17	-31%	+19%	+11%		60
A4	T	-44								
		0	-8	+50	+66	+115%				
							-30%	-16%		n.a.
A5	K	-26								
		+64	--	-38	-300					
						-72%	+39%	-8%		<37
A6	K	-28								
		-27	0	+13	+33	-100%				
							+8%	+6%		<37
A7	T	-9	0	0	+11	+17	+1709%			
							-8%	+4%		72
A8	T	-66								
		-63	-84							
				+200						

			+300				
				+252%			
					+3%	-4%	<37
A9	T	-67					
		-67	-69				
			+113				
			+200				
				+2056%			
					+7%	0%	123
A10							
	T	-47					
		-62	-- +7	+25	+2152%		
					-27%	6%	<37

T = Product from example 1
K = Product from example 2
E = erythema, S = scaling, EX = exudation
SB = subjective improvement, J= improvement in itching

CLM What is claimed is:

1. A method for treating a skin disease in a patient, comprising administering intravenously to a patient suffering from a skin disease an effective amount of an emulsion comprising at least one polyunsaturated long-chain omega-3 or **omega-6 fatty acid**, or a pharmaceutically acceptable ester or salt thereof, and a conventional additive.

2. A method according to claim 1, wherein the fatty acid contains 18-22 carbon atoms.

3. A method according to claim 1, wherein (a) the **omega-3 fatty acid** is selected from .alpha.-**linolenic acid**, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DCHA); and (b) the **omega-6 fatty acid** is selected from **linolenic acid**, .gamma.-**linolenic acid**, dihomo-.gamma.-**linolenic acid** or arachidonic acid.

4. A method according to claim 3, wherein the **omega-3 fatty acid** is eicosapentaenoic acid.

5. A method according to claim 1, wherein (a) the **omega-3 fatty acid** is present in a form selected from a fish oil, a highly purified fish oil concentrate or a linseed oil; and (b) the **omega-6 fatty acid** is present in a form selected from primrose oil, borage oil or soybean oil.

6. A method according to claim 1, wherein the ester is an ethyl ester or a glyceride ester.

7. A method according to claim 6, wherein the ester is a triglyceride.

8. A method according to claim 6, wherein the omega-3 or **omega-6 fatty acid** ester or salt is present in an amount of 4 to 45% by weight in the emulsion.

9. A method according to claim 8, wherein the omega-3 or **omega-6 fatty acid**, ester or salt is present in an amount of 10 to 30% by weight.

10. A method according to claim 8, wherein the emulsion further comprises phospholipids of vegetable or animal origin as emulsifiers.

11. A method according to claim 10, wherein said emulsifier is

ovolecithin.

12. A method according to claim 10, wherein said emulsifier is present in an amount of 5 to 15% by weight based on the total fat content.

13. A method according to claim 1, wherein said emulsion is administered in combination with at least one added ingredient or therapy selected from the group consisting of a retinoid, a cignolin, phototherapy, balneophototherapy, a topical corticosteroid, a parenteral corticosteroid, an oral corticosteroid, a non-steroidal antiphlogistic and an antihistamine.

INCL INCLM: 514/560.000
NCL NCLM: 514/560.000
IC [6]
ICM: A61K031-20
EXF 514/560
ARTU 124
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 17 OF 33 USPATFULL
AN 96:65719 USPATFULL
TI Process for extracting lipids with a high production of long-chain highly unsaturated fatty acids
IN Kohn, Gerhard, Nieder-Olm, Germany, Federal Republic of
Sawatzki, G unther, M unzenberg, Germany, Federal Republic of
Erbe, J urgen, Stuttgart, Germany, Federal Republic of
Schweikhardt, Friedrich, Friedrichsdorf, Germany, Federal Republic of
PA Milupa Aktiengesellschaft, Germany, Federal Republic of (non-U.S. corporation)
PI US 5539133 19960723 <--
WO 9325644 19931223 <--
AI US 1994-185808 19940228 (8)
WO 1993-EP1334 19930527
19940228 PCT 371 date
19940228 PCT 102(e) date
PRAI DE 1992-4219360 19920612
DT Utility
FS Granted
REP EP 92085 Oct 1983
EP 459744 Dec 1991
DE 3213744 Nov 1992
GB 2098065 Nov 1982
REN Patent Abstracts of Japan, vol. 009, No. 071, Mar. 30, 1985.
Patent Abstracts of Japan, vol. 011, No. 232, Jul. 29, 1987.
Database WPI, Section Ch, Week 9043, 1990.
Database WPI, Section Ch, Week, 7404, 1974.
EXNAM Primary Examiner: Dees, Jos e G.; Assistant Examiner: Carr, Deborah D.
LREP Bacon & Thomas
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
AB In the method of the invention to obtain lipids with a high proportion of long-chain polyunsaturated fatty acids (LCPs) with 20 to 22 carbon atoms by extraction from a raw material of animal or vegetable origin, unicellular algae (microalgae), macroalgae from the families of the brown, red and green algae and/or residues of alginate or carrageenin production with a water content of .ltoreq.50 weight % and a particle size of .ltoreq.50 mm are used. For extraction, an organic solvent or a compressed gas is used. A lipid extract with a high proportion of w6 LCP and w3 LCP and in particular with a content of at least 5 weight % of arachidonic acid and/or a content of at least 3 weight % of docosahexanoic acid is also prepared.

PARN This application is a 371 of PCT/EPQ3/01334 filed May 27, 1993.

SUMM This application is a 371 of PCT/EPQ3/01334 filed May 27, 1993.

BACKGROUND OF THE INVENTION

The invention relates to a method for obtaining lipids with a high proportion of long-chain highly unsaturated fatty acids, with from 20 to 22 carbon atoms, by extraction from a raw material of animal or vegetable origin, and to the extract obtained and its use.

1. Field of the Invention

Our foodstuffs include not only saturated fatty acids but also monounsaturated and polyunsaturated fatty acids, which thus have at least one double bond in their carbon chain. These polyunsaturated fatty acids are often designated by abbreviations. The number of carbon atoms or the chain length is given first. This is followed by a hyphen or colon, which in turn is followed by a number that indicates how many double bonds there are in the carbon chain. Following that but separately, the number of omega-carbon atoms is given, counted from the methyl end of the chain, after a "w" or "n". In this system, the short formula for linoleic acid is 18-2 n6.

In fatty acid metabolism in the human being, double bonds are known to be introduced into the carbon chain of a saturated fatty acid. However, this desaturation is possible only after the carbon atom C9 in the direction toward the carboxyl end. The result is that fatty acids such as linoleic acid (18:2 n6) and .alpha.-**linolenic acid** (18:3 n3) must be considered essential, since they cannot be synthesized by the human organism itself but rather must be supplied from food.

From these essential C18 fatty acids, the healthy human organism is capable of synthesizing a number of polyunsaturated fatty acids having from 20 to 22 carbon atoms, by means of further desaturation and chain elongation. The elongation occurs at the carboxyl end of the molecule, and the desaturation occurs between the carboxyl group and the first double bond that follows it. The number of carbon atoms between the methyl end of the fatty acid and the last double bond (omega-C atoms) remains unchanged thereby, so that from linoleic acid (18:2 n6) in lipid metabolism, only **omega-6 fatty acids** (w6 family) are derived, and from .alpha.-**linolenic acid** only-**omega-3 fatty acids** (w3 family) are derived. The course of biosynthesis of the w6 family thus begins with linoleic acid (C 18-2 n6) and proceeds through gamma-**linolenic acid** (C 18-3 n6), di-homo-gamma-**linolenic acid** (C20-3 n6), and arachidonic acid (C20-4 n6) to docosapentanoic acid (C22-5 n6). With respect to the w3 family, the course of biosynthesis begins with .alpha.-**linolenic acid** (C18-3 n3), through octadecatetraenoic acid (C18-4 n3), eicosatetranoic acid (C20-4 n3), eicosapentanoic acid (C20-5 n3) to docosahexanoic acid (C22-6 n3).

By international convention, this group of fatty acids with extraordinary physiological importance is known as LCPs (for long-chain 12olyunsaturated fatty acids). These fatty acids with 20 to 22 C atoms are derived from the essential C18 fatty acids and have at least two double bonds in the acyl radical. The abbreviation LCP will be used below for this group of fatty acids, and a distinction is made between w6 LCPs and w3 LCPs.

The LCPs have versatile biological effects. For instance, they are an indispensable component of all the cell membranes in the body. A change in the membrane lipid composition can cause a great variety of physiological problems.

In recent years, the eicosanoids (prostaglandins, leukotrienes, prostacyclins and thromboxanes) synthesized in the organism from some LCPs have gained particular attention. It has been demonstrated that this highly active group of eicosanoids, in low concentrations, is involved in a number of physiological processes.

In infants and children, in comparison to adults, because of the relatively high need for growth and low reserves, the danger exists of a deficiency in these LCPs. In the last trimester of intrauterine fetal development and during postnatal development of the newborn, large amounts of w6 and w3 LCPs are accumulated in the organs. The capacity for synthesis of the LCPs from the essential precursors appears limited in the young infant, however, because of immaturity of the desaturating enzyme system.

2. Description of the Prior Art

Since these LCP fatty acids are virtually entirely absent from infant formulas previously available, formulas have recently been developed that are enriched with these fatty acids; see German Patent Disclosure DE 39 20 679 A1, for instance.

Because of the increased interest in LCPs, there has been an increased demand for sources of raw materials for such long-chain polyunsaturated fatty acids. The oils containing LCPs that are currently available are quite predominantly obtained from marine cold water fish (see European Patent Disclosure EP 0 292 846 A2 and German Patent Disclosure DE 39 40 239 A1). Such oils from the muscle tissue or organs of fish are distinguished by high proportions of w3 LCPs and in particular of eicosapentanoic acid (20-5 n3) and docosahexanoic acid (22-6 n3). Such oils and particularly oils from fish organs have the disadvantage, however, of a high cholesterol content and also a high content of fat-soluble vitamins and possibly fat-soluble pollutants (heavy metals and pesticides).

It has also already been proposed that LCPs be obtained from autotrophically or heterotrophically fermented microorganisms (see International Patent Disclosures WO 91/07498 and WO 91/119 182 and German Patent Disclosure DE 34 46 795 A1).

The LCPs of interest here can moreover be obtained from organ fats of livestock (cattle/pigs) and from the yolk of chicken eggs (EP 0 074 251 B2). The extraction of LCPs from human placentas is described in EP 0 140 805 A1.

SUMMARY OF THE INVENTION

The object of the present invention is to furnish a method for obtaining LCP-rich lipids from a raw material not previously used for these purposes. The object of the invention is also to furnish a lipid extract or lipid extract fractions that are rich in LCP fatty acids and that provide a foundation for producing foods, in particular baby foods, among others.

This object is obtained by the teaching of the claims.

An essential aspect of the invention is that a certain raw material is used to obtain LCP-containing lipids.

In the method of the invention, one can for instance use macroalgae, primarily occurring in the sea, from the families of brown, red and green algae. Of these, those from the Phaeoophyceae and Rhodophyceae families are of special interest. However, certain species are also used for human nutrition in other parts of the world, above all in the coastal countries of Northern Europe and East Asia (Japan). These macroalgae can be found in many continental shelf zones of the ocean and are available in practically unlimited quantities. A few macroalgae species are also intentionally cultivated in partitioned-off areas of the sea (aquaculture).

It has now surprisingly been found that lipids with a high proportion of LCPs can be extracted from these macroalgae in an economical way, if an organic solvent or a condensed gas is used. Moreover, the macroalgae are comminuted, in particular ground, before the actual extraction, so that the raw material obtained from these macroalgae and used in the method of the invention has a particle size of .ltoreq.50 mm. Furthermore, the macroalgae are dried either before or after the comminution, so that their water content amounts to .ltoreq.50 weight %.

Partially dried products of ground native algae available on the market ("algae flour") can also be used as raw material based on brown and red algae. These commercially available products are available and inexpensive on the market and were previously used only for soil improvement or as an additive to animal feed.

From various brown and red algae species, alginates and carrageenans are currently obtained on a large scale; they are used in the most versatile ways as hydrocolloids in the food industry. To obtain the hydrocolloids, the algae are "planted" or cultivated, harvested, dried and ground on a large scale as described above. Depending on the desired properties of the hydrocolloids to be extracted, different algae species are intentionally mixed and finally extracted in aqueous form.

It has also been surprisingly discovered that the residues that occur in the hydrocolloid extraction, which currently are not used at all or are used to only a very slight extent to produce products for soil improvement or as additives for animal feed, can be used as raw material for the method of the invention. It has in fact been demonstrated that in the extraction to obtain the hydrocolloids using acids and lyes, the LCP-containing lipids present in the algae are not damaged. On the contrary, by the removal of the hydrocolloids from the algae mixtures, there is in fact an improved yield of the extractable lipiris, including the long-chain polyunsaturated fatty acids of interest here, and at the same time there is a lower presence of hydrocolloids and pigments in the extracts.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is preferred, according to the present invention, to use the residues that occur in hydrocolloid production as a raw material used for human nutrition. This provides optimum utilization of the residue of algae extraction. The order of the extraction processes (extraction with an

aqueous and organic solvent) is in principle not significant. However, a prior alginate production is preferred, because following it the relative lipid content in the raw material and thus the yield can be increased, and the presence of hydrocolloids in the lipid extracts is minimized.

As raw material that can be used in the method of the invention, microalgae, which have in the past already been used occasionally for human food in some countries, can also be used according to the invention. These predominantly single cell algae, whose habitat is in fresh water, sea water, or brackish water, are often cultivated in open-field ponds, utilizing sunlight.

In recent years, there have been increasing attempts to ferment single cell algae under defined culture conditions. This algae biomass produced for fermentation is already available on the market, for instance for the species of the Spirulina, Dunaliella and Porphyridium genuses. In addition to these autotrophically cultivated species--that is, species cultivated under sunlight or under artificial light--methods have been developed for producing certain microalgae biomass heterotrophically in closed fermenters at economical cost. All these microalgae from the Cyanophyta, Chrysophyta, Dinophyta, Euglenophyta, Rhodophyta and Chlorophyta phyla, cultivated in open ponds or autotrophically or heterotrophically fermented, can be used according to the invention.

If the residues used according to the invention from hydrocolloid extraction and the microalgae used according to the invention have a moisture content of more than 50 weight % and/or a particle size of more than 50 mm, then this raw material is dried and/or ground prior to the extraction according to the invention, so that the water content of the biomass used in the method of the invention is .ltoreq.50 weight % and the particle size is .ltoreq.50 mm.

A raw material with a water content of from 5 to 50 weight %, in particular 5 to 15 weight %, and a particle size of 0.01 to 50 mm, in particular 0.1 to 1.0 mm, is preferably used.

An organic solvent or a compressed gas is used as the solvent for extraction of the lipids with a high proportion of LCPs. In particular, classic organic solvents and low alcohols with 1 to 6 carbon atoms are used as the organic solvent. Preferably, solvents and alcohols that are miscible with water in any ratio are used. Ethanol can be named as a preferred example. Naturally mixtures of the solvents named may also be used. Preferably, organic solvents that are permitted by the various legal regulations for foods are employed.

As compressed gases, carbon dioxide or propane or a mixture thereof are preferably used. The gas used must be under sufficient pressure and have sufficient temperature to assure that it will be in a liquid or supercritical state. Such compressed gases are characterized by characteristic dissolution properties, particularly for lipophilic ingredients. To change the extraction properties, other gases or liquids may be admixed with the compressed gas as an entraining agent in a quantity such that the mixture under the extraction conditions is in a uniform liquid or supercritical state. As the entraining agent, a compressed gas that is more-polar or more-nonpolar than the compressed gas used for the extraction, or an organic solvent, may be employed. In this way, the polarity of the extraction agent and thus the dissolution properties thereof can be varied or adjusted.

The extraction with an organic solvent is carried out in particular at a

temperature of from 20.degree. C. to 65.degree. C., with the upper temperature value naturally depending on the solvent used. Extraction is preferably done at a temperature of approximately 60.degree. C., especially if ethanol is used as the organic solvent.

The extraction is preferably carried out in the form of a batchwise extraction (maceration), percolation, decanter extraction, or countercurrent extraction. The total yields in these types of method may admittedly be less than in an extraction of all the lipids, for instance with the aid of the exhaustive Soxhlet extraction. However, these procedures can be carried out in substantially less time and are thus more economical.

In extraction with a compressed gas, percolation is preferably carried out.

Although in the context of the present application the term organic solvent is used in the method of the invention, it is nevertheless understood to mean both the corresponding water-free solvents and solvents that contain water (up to 30 volume %). Hence, standard solvents can be used without requiring them to be dried beforehand. Nevertheless, a high proportion of water can impair the yield of the desired lipids.

To separate the lipid extract from the extraction liquid (miscella) obtained from extraction with organic solvents, the temperature of the miscella is preferably lowered enough that the lipid extract precipitates out at least in part. If a solvent that is miscible with water in any ratio is used as the solvent, an example being ethanol or isopropanol, then the lipid extract can also be separated by increasing the water content, and this provision can also be combined with the described lowering of temperature. The organic solvent is then not removed, or is removed only partially, from the miscella. The miscella is mixed with water in a first stage, optionally while being cooled, so that the dissolution capacity of the mixture at the temperature selected is no longer sufficient to keep the lipids in solution. The lipids are then separated from the miscella, for instance with a filter separator.

The liquid solvent can be removed from the resultant solution at standard pressure and high temperatures, and the remaining extract can thus be recovered.

In this separation of the lipid extract from the miscella by increasing the water content and/or by lowering the temperature, the water content of the miscella is preferably increased to 20 volume % to 90 volume %, and in particular to 30 16 50%. The temperature of the miscella is preferably lowered to values of from +20.degree. C. to -60.degree. C, and in particular to +5.degree. C. to -18.degree. C. It is self-evident that the water content of the solvent originally used, and hence of the miscella, was above the values given prior to the addition of the water.

A solvent (such as ethanol) that has a water content of 0 to 20 volume percent, in particular 4 to 15 volume percent, is preferably used.

The situation is similar for the temperature. Hence the temperature of the miscella prior to the lowering of temperature must naturally be above those values to which the temperature is lowered. Preferably the miscella is at a temperature of from 40.degree. C. up to the boiling temperature of the solvent used.

Naturally, the total extract can also be recovered from the miscella by evaporating off the solvent. However, by increasing the water content

and/or lowering the temperature it is possible for the lipids with the fatty acids of interest here to be precipitated out virtually quantitatively.

From the miscella obtained with the aid of an organic solvent or from the extract obtained from that and freed completely or partially of solvent, extraction can be done once again with a compressed gas, preferably carbon dioxide. In this preferred embodiment, the extract obtained with the aid of the organic solvent was fractionated into a nonpolar high triglyceride-containing fraction and a polar phospholipid-containing fraction, which optionally after suitable refinement can be used for manifold purposes.

The conditions for the fractionating extraction with the compressed gas are the same as for the extraction with this gas of the algae etc. originally used.

It was surprisingly found that it is possible, by selection of certain raw materials and by certain extraction steps, to obtain a lipid extract or lipid extract fractions with lipids that are rich in certain LCPs. The subject of the invention accordingly also includes a lipid extract or a lipid extract fraction with a content of arachidonic acid of at least 5 weight % and/or with a content of docosahexanoic acid of at least 3 weight %, in terms of the total weight of the fatty acids. It is self-evident that these fatty acids are not available in free form but rather in "bound form" such as triiglyceride, glycolipid, phospholipid, etc. By furnishing this lipid extract or these lipid extract fractions, it is possible among other things to furnish a raw material for producing baby foods that contains the arachidonic acid and/or docosahexanoic acid important for the development of the child. Lipids that are rich in these fatty acids are otherwise obtainable only with difficulty or in a commercially unsatisfactory way. The content of arachidonic acid and docosahexanoic acid and in particular the content of these two fatty acids in a lipid extract naturally depends on the choice of raw material used. However, it is sufficient if the lipid extract contains one of these fatty acids in a high proportion, since it can naturally be mixed with other extracts and other ingredients.

The subject of the invention is also a lipid extract or a lipid extract fraction obtainable by the method of the invention.

The LCP-containing lipid extracts or individual lipid fractions (triglycerides, glycolipids, phospholipids, etc., or mixtures of both) according to the invention may, optionally after conventional refinement and stabilization, be used as an additive to the fat content of infant formulas. The term "infant formulas" is understood here to include not only the usual starting milk formulas for premature and term infants, but also special products that are offered for therapy or prevention of atopic diseases, for instance.

Because of the characteristic proportions of LCP and their emulsifying properties, phospholipid-containing fractions from algae raw materials in particular, after suitable refinement and stabilization, can also be used as an additive in fat emulsions for parenteral nutrition.

The lipid extracts and/or lipid fractions mentioned, and/or the alkyl esters obtained after hydrolysis and reoesterification of the LCPs, may be used in suitable form (such as gelatin capsules) for the prevention of arteriosclerotic diseases and of **inflammatory** autoimmune diseases.

The LCP-containing fractions and in particular the phospholipid

fractions can serve as an active ingredient additive to cosmetic preparations or as starting material to form liposomes, which can also be added to cosmetic preparations.

Particularly the LCP-containing phospholipid fractions, because of their physical-chemical properties, can be used as emulsifiers in the food and cosmetic industries.

Highly purified fractions of the LCP-containing lipids and the free fatty acids and fatty acid esters obtained after hydrolysis and optional re-estefification can be employed as comparison substances (standards) in analysis.

DETD EXAMPLE 1

Extraction of Various Raw Materials Using Soxhlet Extraction

A number of different algae species and a number of different residues of alginate and carrageenan production were extracted with the aid of exhaustive Soxhlet extraction, using ethanol (96%; V/V) over a period of 40 hours. The lipids were extracted quantitatively. The natural mixtures of triglycerides, glyco- and phospholipids and fat-soluble pigments and vitamins have been referred to as complete lipids hereinafter.

The results of these extractions are summarized in Tables 1 and 2. These tables show the respective fatty acid pattern or the various total lipid or fatty acid yields.

The results listed in these Tables 1 and 2 show that the brown and red algae or the residues of alginate and carragheenin production, extracted according to the invention, have a high content of physiologically significant polyunsaturated fatty acids. Arachidonic acid (AA; 20-4 n6) and docosahexanoic acid (DHA; 22-6 n3) can be named primarily as indicative components. These fatty acids may be extracted in quite different proportions from the various raw materials. While arachidonic acid can be extracted from all raw materials in proportions of 5 to 8 weight %, the raw materials of alginate and carrageenans production designated as alginate 3 and 4 are above all characterized by characteristic docosahexanoic acid contents. In the other raw materials, this fatty acid does not occur, or occurs in only very low proportions.

The extraction yields shown in Table 2 demonstrate that the total extractable lipid content of the various algae raw materials ranges between approximately 10 and 70 g/kg of dry composition, and in each case approximately 50% total fatty acids can be recovered. In the residues of alginate production, the proportion of extractable indicative fatty acids (n6-LCP+n3-LCP) ranges up to 7 g/kg of dry substance, and in the native algae up to 6 g/kg of dry substance.

The method according to the invention can be carried out on a commercial scale especially if the two residues alginate 2 and alginate 3 of alginate production, the single-cell oil (microalgae) and the algae flour from Ascophyllum nodosum are used as raw materials in the method of the invention.

The extract obtained by the solvent extraction can be fractionated and purified by conventional processes. Extraction according to the invention with a compressed gas can also ensue.

TABLE 1

Fatty acid pattern of microalgae, macroalgae, and residues of alginate production
by means of ethanol-Soxhlet extraction in weight % of the total fatty acid pattern

Name	Micro	Alginate 1	Alginate 2	Alginate 3	Alginate 4	Fucus	Asco.	Lam.	Macro.
12-0	0.50	0.78	0.84	0.20	1.06	0.08	0.15	0.51	0.32
14-0	0.49	9.37	6.94	10.04	6.16	11.76	10.40	7.06	13.90
t14-1 n5	0.06	0.27	0.25	0.27	0.15	0.08	0.08	0.24	0.15
r14-1 n5	0.00	0.11	0.09	0.23	1.66	0.09	0.12	0.05	0.82
15-0	0.42	0.51	0.30	0.41	0.55	0.39	0.33	0.43	0.74
16-0	49.66	23.53	17.39	13.40	27.92	19.27	12.40	22.39	29.90
t16-1 n7	3.97	0.25	0.21	0.19	1.33	0.12	0.12	0.00	2.55
16-1 n7	0.45	3.08	3.00	1.45	3.46	1.49	1.28	4.05	3.16
17-0	0.39	0.16	0.11	0.18	0.25	0.15			

0.17
0.14
0.23
18-0 2.97
0.97 0.63 0.66 1.80 0.82
0.93
1.11
1.51
t18-1 n9
0.06
0.03 0.03 0.00 0.23 0.00
0.02
0.02
0.14
18-1 n9
1.30
29.34 22.64 44.34 22.92 36.33
43.80
20.01
22.40
18-2 n6
6.94
7.37 6.15 7.44 3.32 8.74
8.00
5.83
3.87
18-3 n3
0.78
3.40 5.69 1.74 0.78 2.64
2.30
6.00
2.00
18-4 n3
0.00
3.31 11.27 1.00 1.40 2.15
1.43
9.92
2.03
20-0 0.00
0.75 0.43 0.32 2.00 0.43
0.29
0.49
1.13
20-1 n9
0.00
0.04 0.05 0.09 0.80 0.05
0.11
0.02
0.04
20-2 n6
1.09
0.29 0.51 1.25 0.15 0.22
1.37
0.14
0.04
20-3 n6
0.98
0.45 0.41 0.58 0.48 0.76
0.59
0.30
0.33
20-4 n6
12.06
7.20 8.41 5.22 5.01 7.34
8.05
7.72

									7.85
20-3 n3									
	0.00								
		0.08	0.16	0.03	0.02	0.06			
							0.27		
								0.14	
									0.03
20-5 n3									
	8.19								
		5.54	10.62	1.87	1.18	3.15			
							3.06		
								9.82	
									2.41
22-0 0.00									
		0.08	0.04	0.15	0.03	0.15			
							0.12		
								0.02	
									0.09
22-1 n9									
	0.00								
		0.01	0.01	0.14	0.68	0.02			
							0.30		
								0.37	
									0.04
24-0 0.00									
		0.09	0.11	0.21	0.00	0.14			
							0.20		
								0.05	
									0.20
24-1 n9									
	0.00								
		0.55	0.35	1.70	0.01	0.89			
							1.19		
								0.02	
									0.11
22-6 n3									
	0.00								
		0.03	0.00	3.56	7.40	0.17			
							0.08		
								0.10	
									0.05
n.i.		9.69	3.38	3.34	9.24	2.50			
							2.74		
								3.05	
									3.85
trans FS									
	4.63								
		0.55	0.49	0.46	1.72	0.2	0.22		
								0.26	
									2.84
TOTAL									
	100	100	100	100	100	100	100	100	
									100

Micro = microalgae, such as Porphyridium cruentum; Alginates 1-4 = variou residues from alginate production; Fucus = Fucus serratus; Asco. = Ascophyllum nodosum; Lam. = Laminaria digitata; Macro. = Macrocystis pyrifera; n. i. = not identified; trans FS = transfatty acids.

TABLE 2

Total extract, lipid extract and fatty acid yields of microalgae, macroalgae and residues of
alginate production from ethanol-Soxhlet extraction in g/kg of raw material dry substance

Micro

	Micro	Alginate 1	Alginate 2	Alginate 3	Alginate 4	Lam.	Macro.	Fucus	Asco.
GE	233.7	77.22	98.25	111.32	52.28	130.64	61.68	246.92	157.05
LE	20.7	55.60	73.51	60.36	22.16	22.45	11.25	46.15	73.74
Ges.-FS	10.1	28.07	34.81	34.00	7.54	9.78	5.11	27.26	42.19
% FS in LE	48.8	50.49	47.36	56.33	37.48	43.59	45.40	59.07	57.20
n6-LCP	14.14	2.28	3.36	2.48	0.47	0.82	0.44	2.33	4.34
n3-LCP	8.19	1.62	3.88	1.92	0.71	1.02	0.13	0.95	1.48
20-4 n6	2.49	2.07	3.03	1.84	0.42	0.78	0.42	2.05	3.49
20-5 n3	1.69	1.59	3.83	0.66	0.10	0.99	0.13	0.88	1.33
22-6 n3	0.00	0.01	0.00	1.25	0.61	0.01	0.00	0.05	0.03

Micro = microalgae, such as Porphyridium cruentum; Alginates 1-4 = variou residues from alginate production; Lam. = Laminaria digitata; Macro. = Macrocystis pyrifera; Fucus = Fucus serratus; Asco. = Ascophyllum nodosum GE = total extract; LE = lipid extract after removal of the hydrophilic ingredients ("FOLCH"); Ges. FS = total fatty acids; n6LCP = total of n6

fatty acids with 20 and more carbon atoms in the acyl radical; n3LCP = total of n3 fatty acids with 20 and more carbon atoms in the acyl radical 204 n6 = arachidonic acid; 205 n3 = eicosapentanoic acid; 226 n3 = docosahexanoic acid.

The extract is preferably freed of solvent completely by distillation at reduced pressure, and then subjected to the usual purification processes in the production of food fats in industry, such as bleaching, degumming and deodorization. The bleaching agent can already be added before complete removal of the solvent has occurred.

EXAMPLE 2

Extraction of Lipids From the Alga Ascophyllum nodosum With 90% Ethanol

Flour of the alga Ascophyllum nodosum was used as the raw material.

Characterization of the Ascophyllum nodosum raw material:

Water content: 9.6%

Particle size Screen mesh width (mm)	% distribution
0.5	0.2
0.25	0.4
0.1	75.4
0.05	23.1
>0.05	0.9

The solvent extraction over four hours was performed with ethanol (90%; V/V) at various temperatures. In the apparatus used, the process was a single-stage percolation done on a laboratory scale. The ratio between the extraction agent used and the raw material dry substance was approximately 4:1 (weight/weight).

In comparison with the Soxhlet extraction (see Table 3), in which an extraction time of 40 hours was adhered to, the yield of the four-hour percolation was uniformly quite high. It demonstrated that increasing the extraction temperature leads to an improved yield in all the extract ingredients. Further optimization of the yield in the extraction of this raw material is attainable by further increasing the ratio between the solvent and the raw material or by changing the course of the process in the direction of countercurrent extraction (see Example 3).

TABLE 3

Extraction yields in percolation, in percent of raw material dry substance Temp. (.degree.C.)		20	30	40	50	60	Soxhlet
GE	%	8.06	11.01	14.34	16.54	17.89	15.7
FS	%	0.93	1.18	1.25	1.34	1.41	4.22

20-4 n6	%	0.09	0.11	0.12	0.13	0.14	0.35
20-6 n3	%	0.05	0.06	0.06	0.07	0.08	0.13
Concen-tration	%	2.5	2.3	3.1	3.4	3.7	

GE = total extract; FS = fatty acid yield; 204 n6 = yield of arachidonic acid; 205 n3 = yield of eicosapentanoic acid

The results of the one-stage percolation test shown in FIG. 3 show that with this experimental apparatus, yields of 41% of the arachidonic acid fractions and 58.5% of the eicosapentanoic acid fractions are attained, compared with the Soxhlet extraction. By the percolation process, it was possible to recover a total of 33.4% of the maximum extractable fatty acids. The value for the total extract yield, which is elevated compared with Soxhlet extraction, can be ascribed to the higher water content (10%) of the ethanol used in the percolation and the resultant more-intensive extraction of hydrophilic substances.

From Table 3, it is also clear that for the extraction of the flour of the alga Ascophyllum nodosum with 90% ethanol, an extraction temperature of approximately 60.degree. C. is to be sought, if the method is to be optimized in terms of the miscella concentration, that is, the solvent consumption. Naturally comparable yields are also attainable at lower temperatures and with a correspondingly higher amount of solvent.

EXAMPLE 3

Staged Countercurrent Extraction of a Residue From Alginate Production With 90% Ethanol

In this example, a raw material was used that was already used to recover alginate (alginate 1). This residue is a mixture of various species of brown algae. The algae residues were dried and ground after the alginate production process, so that in terms of particle size and water content they are approximately equivalent to the flour of the alga Ascophyllum nodosum (see Example 2).

The extraction method of staged countercurrent extraction employed is equivalent to the process carded out on an industrial scale. In contrast to percolation (Example 1), the extract concentration of the miscella in multistage countercurrent extraction fluctuates around a mean value. The more stages that are used and the shorter the dwell time of the raw material in the apparatus, the less pronounced this fluctuation is. With this method, by comparison with percolation, identical yields can be attained, using only a fraction of the solvent, or higher yields can be attained using similar quantities of solvent.

In the example chosen, a four-stage experimental apparatus was chosen. The extraction temperature was 20.degree. C.; the ratio between the quantity of extraction agent used and the raw material dry substance was 2:1.

Despite the comparatively simple apparatus (30 stages and more are not unusual in industrial systems), it was possible in this example to attain yields of 66% of arachidonic acid, 72.5% of eicosapentanoic acid and 45.7% of the total fatty acids, by comparison with the Soxhlet extraction.

TABLE 4

Yields of the multistage countercurrent extraction of alginate 1 compared with Soxhlet extraction, in weight % in terms of the raw material dry substance

Yield	Countercurrent extraction	Soxhlet extraction
Total extract	5.92	7.72
Total lipid extract	4.24	5.56
Total fatty acids	1.28	2.80
Arachidonic acid (20-4 n6)	0.13	0.20
Eicosapentanoic acid (20-5 n3)	0.12	0.16

If these values are compared with the results of the percolation in Example 2, the advantages of countercurrent extraction become clear. Although in countercurrent extraction only 50% of the quantity of solvent was used and the extraction temperature was only 20.degree. C., the yields in terms of total fatty acid and arachidonic acid are nevertheless clearly superior to that of percolation.

EXAMPLE 4

Extraction of Flour of the Alga Ascophyllum nodosum with Compressed Carbon Dioxide

For extraction with compressed gases, the dried, ground raw material of native algae or of residues from alginate and carrageenan production is used. The water content of the starting material is typically between 5 and 50 weight % and preferably between 10 and 20 weight %. The particle size of the material is 0.01 mm to 50 mm, preferably 0.1 mm to 0.3 mm.

The raw material used is the algae flour of Ascophyllum nodosum already employed in Example 2. The extraction was carried out with compressed carbon dioxide (150 bar, 35.degree. C., approximately 11 kg of CO.sub.2 /kg of raw material).- Under these conditions, at least 90% of the total lipids extractable with this solvent can be recovered.

TABLE 5

Extract and fatty acid yields of high-pressure extraction of Ascophyllum nodosum in comparison with ethanol Soxhlet extraction, in weight % of the raw material dry substance

Yield	High pressure extraction	Soxhlet extraction
Total lipid extract	2.50	7.30
Total fatty acids	2.10	4.20
Arachidonic acid (20-4 n6)	0.18	0.35
Eicosapentaenoic acid (20-5 n3)	0.08	0.13

In Table 5, the maximum attainable yields in terms of the raw material mentioned with each method are compared. Soxhlet extraction here stands for processes of solvent extraction using ethanol overall because in

principle this represents nothing more than a repeated process of percolation/maceration.

To enable evaluating the quality of the extracts obtained, the fatty acid pattern of the extracts recovered by the two methods are compared in Table 6.

TABLE 6

Comparison of the fatty acid spectra of a high-pressure extract and a Soxhlet extract of Ascophyllum nodosum; figures given as weight % of the total fatty acids

Fatty Acids	High-pressure Extract	Soxhlet Extract
14-0	5.58	10.4
14-1 n5	0.15	0.12
16-0	7.46	12.4
16-1 n7	0.94	1.28
18-0	0.97	0.93
18-1 n9	53.8	43.8
18-2 n6	9.56	8.0
20-0	0.34	0.29
18-3 n3	1.58	2.3
20-1 n9	0.48	0.11
20-2 n6	1.77	1.37
22-0	2.88	0.12
20-3 n6	0.91	0.59
22-1 n9	0.44	0.30
20-4 n6	7.26	8.05
20-5 n3	2.18	3.06

The results in Table 6 clearly show that the solvent extract of Ascophyllum nodosum has only a slightly more favorable composition in terms of the proportion of arachidonic acid and eicosapentanoic acid, compared with the high-pressure extract. Since with the aid of high-pressure extraction, it is primarily nonpolar lipids (triglycerides) that are extracted, it must be presumed that the indicative fatty acids also occur in high proportions in these lipids.

EXAMPLE 5

Separation of the Lipid Fraction From the Miscella by Increasing the Water Content

Separation of the lipid fraction from the miscella by increasing the water content was carried out in the example of the miscella obtained by

Staged countercurrent extraction of alginate 2 with 90% ethanol. Five aliquot portions were taken from the miscella and adjusted to various water contents, beginning with 10% water in the starting miscella (see Table 7). The extracts precipitated after the addition of water were recovered by centrifuging, decanting of the remainder, and ensuing drying.

Table 7 below clearly shows the relationship that exists between the water content of the miscella and the yields of the precipitated lipid fractions. The table shows the lipid yields obtained with a comparison extract that was recovered from by complete evaporation off of the solvent of an aliquot quantity of the starting miscella. The yields are shown relatively in percent of comparison extract (comparison extract =100%).

TABLE 7

Lipid yields from the miscella after precipitation by increasing the water content, in percent of the comparison extract (equals 100%): values for water content in absolute percent

Water Content	25.0	35.7	43.75	50	55
GE	46.06	78.63	84.76	87.31	88.73
LE	48.28	74.92	80.68	84.8	88.32
FS	51.59	90.07	93.24	94.61	84.7
20-4 n6	64.56	100.2	102.9	103.1	97.58
20-5 n3	57.49	97.94	102.32	104.41	95.62

GE = total extract; LE = lipid extract; FS = fatty acids; 204 n6 = arachidonic acid; 205 n3 = eicosapentanoic acid

The values in Table 7 show that increasing the water content of the miscella from 10% to 35% is already sufficient for the lipids, in which the indicative fatty acids arachidonic acid and eicosapentanoic acid are localized, to be virtually quantitatively precipitated out. The values over 100% are suspected to be the result of the fact that because of the greater purity of the lipid fractions after precipitation in comparison with the comparison extract, the fatty acids can be more completely converted into the derivatives that are determinable by gas chromatography.

The method of lipid precipitation from the miscella employed here, by increasing the water content, also has the following advantages in terms of the process:

1. As a result of the major increase in the water content of the miscella, prepurification of the extract takes place, because hydrophilic substances, which at a 10% water content of the extraction agent are already co-extracted, remain in solution and in this way need not be removed from the total lipid extract only afterward by complicated processes.

2. Alginates or carragheenins, which are extracted primarily from native algae in sometimes quite major quantities with 90% ethanol, can cause problems in solvent recovery in modern evaporator systems (such as downflow evaporators). However, if the lipid extract is obtained by adding water from the miscella, then the excess water keeps the hydrophilic hydrocolloids in solution, so that the separation of the solvent from the remaining miscella can be carried out without problems.

In order to check whether a selective precipitation of the lipids from the miscella and hence a variation in the fatty acid pattern of the lipids occurs from the water addition, the extract precipitated out with a miscella water content of 55% and the comparison extract were studied by gas chromatography as an example.

Extract 1 listed in Table 8 below was obtained from the starting miscella by increasing the water content to 55%, while the comparison extract was obtained by evaporating the solvent out of the starting miscella.

TABLE 8

Fatty acid pattern of an extract precipitated out with a 55% water proportion, compared with the total extract:

figures given in weight % of the total fatty acids

Fatty Acids	Extract 1	Comparison Extract
14-0	8.26	7.9
14-1 n5	0.76	0.69
16-0	19.73	18.9
16-1 n7	4.28	4.32
18-0	0.43	0.39
18-1 n9	24.56	22.46
18-2 n6	7.88	7.43
20-0	0.37	0.34
18-3 n3	6.97	6.77
20-1 n9	0.17	0.15
20-2 n6	0.27	0.26
20-3 n6	0.4	0.37
22-1 n9	0.08	0.07
20-4 n6	11.44	10.72
20-5 n3	10.56	10.01
22-6 n3	0.46	0.36

The results of the fatty acid analysis in Table 8 show that the fatty acid spectra of the two extracts are not substantially different. The proportions of the indicative fatty acids arachidonic acid and eicosapentaenoic acid, at 10 to 11 weight %, also virtually agree. This result confirms the findings already shown in Table 7, that by purposeful addition of water a quantitative precipitation of the lipids and fatty acids from the raw miscella is possible.

EXAMPLE 6

The separation of the neutral lipid extract by extraction with compressed gases is described below.

In a suitable system, the miscella obtained with organic solvents (preferably ethanol) or an extract freed completely or partially of the solvent is brought into contact in an extraction autoclave with compressed gases, preferably carbon dioxide. An entraining agent can be added in metered fashion to the compressed gas in order to purposefully adjust the extraction properties.

If the liquid solvent was removed completely from the extract and no entraining agent was admixed with the extraction gas, then the triglycerides, carotenoids, chlorophylls and phytosterols are dissolved selectively out of the extract. By a suitable selection and metered addition of the entraining agent or incomplete separation of the liquid solvent from the extract, the dissolution properties of the extraction gas can be expanded substantially.

By reducing the pressure and/or raising the temperature of the extraction gas, its solubilizing capacity is reduced in stages. In each of these stages, a certain portion of the dissolved lipids occurs as an extract fraction. In this way, fractionation of the lipids into one nonpolar and one polar fraction primarily takes place. The nonpolar lipid fraction can then, in the course of a multistage precipitation, be subjected to further fractionation.

This separation will be described in detail in terms of a CO.sub.2 high-pressure countercurrent extraction of a miscella.

An aliquot of a miscella that was obtained by maceration of alginate 2 with 90% ethanol is extracted in countercurrent with compressed carbon dioxide (150 bar, 35.degree. C.) in a stripper column. After the

extraction, the compressed gas is expanded in a precipitation autoclave to 20 bar, so that the lipid dissolved in the fluid occurs in the form of extract. The fraction not soluble in the fluid collects at the bottom of the column. It comprises polar lipids and the non-lipophilic remaining extract. The fill level of the separator and of the stripper column can be monitored visually during the extraction via viewing windows. In the course of the extraction, when a certain fill level is attained, both fractions can be removed via a valve.

The maceration was carried out in this example as a one-stage batch extraction at 35.degree. C. for 20 hours. The miscella concentration prior to high-pressure extraction was 1.37% in terms of the total extract and 1% in terms of the lipid extract. Some of the miscella obtained by the maceration was removed and used for the high-pressure extraction. After the high-pressure extraction, 41.3% of the total extract and 50.9% of the lipid extract could be found in the separator.

Gas Chromatography Investigation of the Extracts in the Separator and Prior to the High-pressure Extraction

In order to be able to evaluate whether the neutral lipid extract obtained by high-pressure extraction differs in the composition of the fatty acids compared with the total extract of the fractionation, a gas chromatography analysis of the fatty acid pattern of the extract was carried out before and after the high-pressure extraction.

TABLE 9

Fatty acid pattern of a lipid extract of alginate 2, obtained by maceration, before and after high-pressure extraction (HD-E) in weight % of the total fatty acids

Fatty Acids	Before HD-E	After HDE i.S.
14-0	8.25	7.65
t14-1 n5	0.07	0.00
14-1 n5	0.41	0.27
15-0	0.36	0.11
16-0	20.17	19.42
t16-1 n7	0.27	0.23
16-1 n7	3.56	3.42
17-0	0.15	0.11
18-0	1.04	0.61
t18-1 n9	0.07	0.02
18-1 n9	26.02	22.85
18-2 n6	7.10	6.18
19-0	0.00	0.00
18-3 n3	4.68	6.18
18-4 n3	9.64	12.47
20-0	0.61	0.39
20-1 n9	0.14	0.05
20-2 n6	0.49	0.42
20-3 n6	0.50	0.35
20-4 n6	7.84	7.60
20-3 n3	0.14	0.15
20-5 n3	7.68	10.37
22-0	0.05	0.07
22-1 n9	0.11	0.17
24-0	0.07	0.06
24-1 n9	0.10	0.28
22-06 n3	0.11	0.03
Total	100	100

HD-E = highpressure extraction; FS = fatty acids; i.S. = in the separator

Comparison of the fatty acid spectra of the lipid extracts before and after the high-pressure extraction shows that the fatty acid pattern of the neutral lipid fraction does not differ substantially from the total extract. The proportion of the physiologically significant fatty acids arachidonic acid (20-4 n6) and eicosapentaenoic acid (20-5 n3) in the neutral lipid extract does not vary toward the negative as a result of the high-pressure extraction, but in the case of the eicosapentaenoic acid on the contrary even rises.

The results of this experiment show that the LCPs are localized in substantial proportions in the neutral lipids of the residues from alginate production.

CLM What is claimed is:

1. A method for obtaining lipids with a high proportion of long-chain polyunsaturated fatty acids (LCPs) having from 20 to 22 carbon atoms by extraction from a raw material of plant origin, characterized in that as the raw material residues from alginate or carrageenan production which have been subjected to drying to provide a water content of .ltoreq.50 weight % and a particle size of .ltoreq.50 mm are used, and that for extraction an organic solvent or a compressed gas is used.

2. The method of claim 1, characterized in that a raw material with a water content of 5 to 50 weight % and with a particle size of 0.01 to 50 mm is used.

3. The method of claim 1, characterized in that extraction is done with a solvent miscible with water in any ratio, at a temperature of 20.degree. C. to 65.degree. C. and that the extraction is carried out in the form of a batchwise extraction (maceration) percolation, decanter extraction or countercurrent extraction.

4. The method of claim 1, characterized in that the extraction liquid (miscella) obtained with the aid of a solvent miscible with water in any ratio at 20.degree. C. or more is diluted with water and/or cooled down to between +20.degree. C. and -60.degree. C. to such an extent that the lipid extract at least partly precipitates out, and that the thus-obtained lipid extract is separated.

5. The method of claim 1, characterized in that the miscella obtained with the aid of an organic solvent, or the extract obtained from it and partially or completely freed of solvent, is extracted with a compressed gas, and the neutral lipid fractions dissolved in the compressed gas and as desired the polar lipid fraction that has not entered into solution in the compressed gas are isolated.

6. The method of claim 1, characterized in that the extraction is carried out to maintain the pressures of the compressed gases from 60 to 2000 bar and the temperature from -20.degree. C. to +200.degree. C.

7. The method of claim 1, characterized in that carbon dioxide is used as the compressed gas, to which an entraining agent may be added.

8. A lipid extract or lipid extract fraction of residues of alginate or carrageenan production, characterized by a content of arachidonic acid of at least 5 weight % and/or a content of docosahexanoic acid of at least 3 weight %, referred to the total weight of the fatty acids.

9. A lipid extract or lipid extract fraction, obtainable by the method

of claim 1.

10. The lipid extract or lipid extract fraction of claim 9, characterized by a content of arachidonic acid of at least 5 weight % and/or a content of docosahexanoic acid of at least 3 weight %, referred to the total weight of the fatty acids.

11. A food, characterized in that it contains a lipid extract or a lipid extract fraction of claim 8.

12. An additive to the fat body of infant nutrition products, to dietetic products for arteriosclerosis prevention, to dietetic products for the prevention of autoimmune diseases (atopias) and to cosmetic products, consisting essentially of the lipid extract or lipid extract fraction of claim 8.

13. The method of claim 2, characterized in that a raw material with a water content of 5 to 15 weight % and with a particle size of 0.1 to 1.0 mm is used.

14. The method of claim 3, characterized in that the solvent miscible with water is a low alcohol and the extraction is done at a temperature of about 60.degree. C.

15. The method of claim 4, characterized in that the solvent miscible with water is ethanol, and the extraction liquid (miscella) is diluted with water to 20 to 90 volume %.

16. The method of claim 6, characterized in that the extraction is carried out to maintain the pressures of the compressed gases from 70 to 500 bar and the temperature from +20.degree. C. to +60.degree. C.

17. A product produced by the method of claim 1.

INCL INCLM: 554/020.000
INCLS: 554/008.000; 554/224.000
NCL NCLM: 554/020.000
NCLS: 554/008.000; 554/224.000
IC [6]
ICM: C07C001-00
EXF 554/20; 554/8; 554/224
ARTU 124
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 18 OF 33 USPATFULL
AN 96:27183 USPATFULL
TI Enteral nutritional composition having balanced amino acid profile
IN Schmidl, Mary K., Arden Hills, MN, United States
Kvamme, Candis, Brooklyn Park, MN, United States
PA Sandoz Nutrition Ltd., Berne, Switzerland (non-U.S. corporation)
PI US 5504072 19960402 <--
AI US 1995-387038 19950210 (8)
RLI Continuation of Ser. No. US 1993-134226, filed on 8 Oct 1993, now patented, Pat. No. US 5438042
DT Utility
FS Granted
REP

US 3697287	Oct 1972	426/073.000	Winitz
US 3698912	Oct 1972	426/656.000	Winitz
US 3701666	Oct 1972	426/311.000	Winitz
US 4414238	Nov 1983	426/602.000	Schmidl
US 4670268	Jun 1987	426/072.000	Mahmond
US 4737364	Apr 1988	424/195.100	Kalogris

US 4752618 Jun 1988 514/549.000 Mascioli et al.
US 4847296 Jul 1989 514/552.000 Babayan et al.
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US 5053387 Oct 1991 514/002.000 Alexander
US 5221668 Jun 1993 514/023.000 Henningfield et al.
US 5231085 Jul 1993 514/044.000 Alexander et al.
REN Tolerex Product Literature, Norwich-Eaton Pharmaceuticals, Norwich, N.Y.
(1988).
Vivonex T.E.N. Product Literature, Norwich Eaton Pharmaceuticals, Inc., Norwich, N.Y. (1983).
High Nitroten Vivonex Product Literature, Norwich-Eaton Pharmaceuticals (1978).
Vivonex-T-E-N Product Literature, Sandoz Nutrition Corporation, Minneapolis, MN. (1992).
J. W. Alexander et al., Ann. Surg., vol. 192, pp. 505-517 (1980).
L. Dominioni et al., J. Burn Care Rehab., vol. 5, pp. 106-112 (1984).
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Food and Nutrition Board-National Research Council, Recommended Dietary Allowances, 10th Ed., Nat. Acad. of Sciences, pp. 66-67 (1989).
M. Donnelly et al., J. Cell Physiol., vol. 89, pp. 39-52 (1976).
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: Degen, Nancy J.
LREP Honor, Robert S., Battle, Carl W.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
AB Enteral nutritional composition comprising 4-30% lipid component, 65-80%
carbohydrate component and 16-25% protein component, based on total caloric content, wherein said protein comprises by weight 14-30% glutamine and 5-33% arginine and said composition has a nonprotein calorie to grams of nitrogen ratio of 150:1 to 80:1.

PARN BACKGROUND OF THE INVENTION

INFORMATION DISCLOSURE

This is a continuation of U.S. application Ser. No. 08/134,226, filed Oct. 8, 1993, now U.S. Pat. No. 5,438,042.

SUMM In general, enteral nutrition compositions may be administered orally or
by tube feeding. Numerous enteral formulations are utilized in patients with a hypermetabolic state as effected by burns, trauma, surgery and in
patients with malnutrition, chronic illness and in disorders resulting from prolonged periods of reduced oral intake resulting from cerebral vascular accidents or a comatose state.

The enteral compositions have provided benefits and advantages to parenteral nutrition. Elemental diets are often indicated for patients who have a reduced gastrointestinal absorptive surface or exocrine pancreatic insufficiencies. A few of the clinical indications for elemental formulas include: pancreatitis, short gut syndrome, radiation enteritis, GI cutaneous fistulas and Crohn's disease. For some patients,
they may also be useful as a transition feeding or even replace total parenteral nutrition (TPN). This recommendation is based on recent clinical findings that demonstrate elemental diets when compared to TPN result in fewer complications, reduced patient length of stay in the ICU

and are less expensive. Elemental diets are composed of low molecular weight nutrients and require minimal digestive and absorptive capability. The protein source consists of free amino acids and contains essential and non-essential amino acids. Carbohydrate is typically composed of glucose and hydrolyzed cornstarch (maltodextrin) while the fat content is usually low and primarily consists of essential fatty acids. These diets have minimal residue because of the efficient absorption of the nutrients provided in an elemental form. Most practitioners find that initiating feeding at full strength using low delivery rates ,is well tolerated, even though elemental formulas are, by nature, hyperosmolar (greater than 300 mOsm/Kg H.sub.2 O). However, in selected cases, initiating feeding with a dilute formula may be preferred. Elemental diets are often administered by needle catheter jejunostomy or endoscopically placed percutaneous jejunal tubes (PEJ) or nasoenteric small bowel feeding tubes in the critically ill patient.

ISOCAL is an enteral formulation by Mead Johnson which utilizes casein and soy for its protein source, glucose oligosacchrides for its carbohydrate source and soy oil and medium chain triglycerides (MCT) oil for its lipid source.

OSMOLITE is manufactured by Ross and utilizes as its protein source casein and soy, corn starch for its carbohydrate source and fifty percent MCT oil, forty percent corn oil and ten percent soy oil for its lipid source.

ENSURE is manufactured by Ross and utilizes casein and soy for protein source, corn starch and sucrose for a carbohydrate source and corn oil for a lipid source.

SUSTACAL manufactured by Mead Johnson utilizes casein and soy for its protein source, corn syrup and sucrose for its carbohydrate source and soy oil for its lipid source.

ENSURE PLUS manufactured by Ross is a high protein, high calorie composition using soy and casein for its protein source, corn starch and glucose for its carbohydrate source and corn oil for its lipid source.

MAGNACAL manufactured by Sherwood Medical is a high density composition with 2.0 calories/ml. MAGNACAL utilizes casein for its protein source, corn syrup for its carbohydrate source and soy oil for its lipid source.

TRAUMACAL manufactured by Mead Johnson utilizes casein for its protein source, corn syrup and sucrose for its carbohydrate source and 70 percent soy bean oil. and 30 percent MCT oil for its lipid source.

ISOTEIN HN is manufactured by Sandoz and utilizes lactalbumin for its protein source, maltodextrin for its carbohydrate source and soy oil and MCT oil for its lipid source.

VIVONEX T.E.N. is manufactured by Sandoz and comprises branched chain amino acids, glutamine and arginine as the protein source, safflower oil as the lipid source, and maltodextrin and modified starch as the carbohydrate source.

IMPACT is manufactured by Sandoz and comprises arginine and caseinates as the protein source, maltodextrins as the carbohydrate, and menhaden oil and structured lipids as the lipids source.

U.S. Pat. No. 4,752,618 describes a dietary supplement and method of minimizing infections therewith, comprising omega-3 and **omega-6 fatty acid** such as safflower oil and menhaden oil.

U.S. Pat. No. 4,847,296 describes triglyceride preparations for enteral administration. to prevent catabolism and increase protein synthesis in subjects undergoing severe metabolic stress.

U.S. Pat. No. 5,053,387 describes enteral compositions for treating traumatic injury comprising an intact protein (from lactalbumin egg albumen or whey and the like), arginine, carbohydrate (glucose polymers, disaccharides, starches and the like), lipid comprising **omega-3 fatty acids** of fish oil, and necessary vitamins and minerals.

U.S. Pat. No, 5,231,085 describes enteral compositions comprising arginine, ornithine, a nucleobase, omega-3 polyunsaturated fatty acids, and omega-6 polyunsaturated fatty acids.

Other background references on enteral feeding compositions and methods include:

Alexander, J. W., MacMillan, B. G., Stinnett. J. P. et al: Beneficial effects of aggressive protein feeding in severely burned children. Ann. Surg. 192:505 -517, 1980;

Dominioni, L., Trocki, O., Mochizuke, H., et al: Prevention of severe postburn hypermetabolism and catabolism by immediate intragastric feeding. J. Burn Care Rehab. 5:106-112, 1984;

Bower, R. H., Muggia-Sullam, M., Vallgren, S., et al: Branched chain amino acid enriched solutions in the septic patient. A randomized. prospective trial. Ann. Surg. 203: 13-20, 1986;

Cerra, F. G., Siegal, J. H., Coleman, B., et al: Septic autocannibalism: A failure of exogenous nutritional support. Ann. Surg. 192:570-580. 1980;

Cerra, F. B., Upson, D., Angelico, R., et al: Branched chains support post-operative protein synthesis. Surgery 92:192-198, 1982;

Cerra, F. B. Shronts, E. P., Konstantinides, N. N., et al: Enteral feeding in sepsis: A prospective randomized, double-blind trial. Surgery 98:632-638, 1985;

Law, D. K., Durdick, S. J. and abdon, N. I.: The effect of dietary protein depletion on immuno competence: the importance of nutritional repletion prior to immunologic induction. Ann. Surg. 179:168-173, 1974;

Dominioni, L., Trocki, O., Fang, C. H., and Alexander, J. W.: Nitrogen balance and liver changes in burned guinea pigs under going prolonged high protein enteral feeding. Surg. Forum 34:99-101, 1983;

Food and Nutrition Board, National Research Council, Recommended Dietary Allowances, 10th edition. Washington, D.C., National Academy of Sciences, 1989; and

Donnelly, M. and Scheffler, I. E.: Energy metabolism in respiratory deficient and mild type Chinese hamster fibroblasts in culture. J. Cell Physical. 89:39-51, 1976.

Improvements to the enteral feeding compositions are continually sought to provide greater patient benefits. It is the object of the present invention to provide an improved enteral nutritional composition that is nutritionally balanced and which provide complete nutritional support for critically ill patients.

SUMMARY OF THE INVENTION

The present invention relates to an enteral nutritional composition comprising based on total caloric content of said composition,

a) from 4 to 30% lipid component,

b) from 65 to 80% carbohydrate component, and

c) from 16 to 25% protein component, said protein component comprising based on the free base 14 to 30% by weight glutamine and 5 to 33% by weight arginine, said glutamine and arginine being in free base form, ingestible salt form, partially hydrolyzed protein form or intact protein form,

wherein said composition has a nonprotein calorie to grams of nitrogen ratio ranging from 150:1 to 80:1.

The composition can be in the form of a solid powder (which is subsequently mixed with water or other suitable liquid carriers) or in the form of a ready-to-use homogenous aqueous liquid. The composition is useful in the dietary management of stress, trauma, burns, malnutrition, sepsis, **inflammatory** bowel disease, cancer, intestinal atresia, pancreatitis, fistula, short-gut syndrome, acquired immunodeficiency syndrome, cachexia, and other stressed and catabolic conditions.

It is a further object of this invention to provide an improved enteral feeding composition having higher total nitrogen, lower ratio of nonprotein calorie to gram of nitrogen ratio and improved amino acid profile. It is another object of this invention to provide improved enteral feeding composition containing carnitine and taurine and the resultant benefits therefrom. It is an even further object of this invention to provide a method of treating stressed catabolic patients by enteral administration of the improved feeding compositions of this invention.

DETAILED DESCRIPTION

This invention relates to improved enteral nutritional compositions which are useful in treating stressed and catabolic conditions. The enteral nutritional composition of this invention comprises, based on total caloric content of said composition,

a) from 4 to 30% lipid component,

b) from 65 to 80% carbohydrate component, and

c) from 16 to 25% protein component, said protein component comprising based on the free base 14 to 30% by weight glutamine and 5 to 33% by weight arginine, said glutamine and arginine being in free base form, ingestible salt form, partially hydrolyzed protein form or intact protein form,

wherein said composition has a nonprotein calorie to grams of nitrogen ratio ranging from 150:1 to 80:1.

The composition comprises based on total calories from 4-30% (preferably about 6%) lipid component. Adequate fat intake is important as a source of energy, essential fatty acids and carrier of fat soluble vitamins. Relatively low levels of fat (i.e., <3% calories) may be inadequate on a long-term basis (i.e., >2 weeks) to prevent essential fatty acid deficiency. Suitable lipids for use in the present invention include, any of the conventional saturated and unsaturated fatty acids, glycerides and other nutritionally acceptable fat sources known in the art, such as animal oils, fish oils, vegetable oils and synthetic lipids. Lipid sources include, for example, medium chain triglycerides, corn oil, soybean oil, peanut oil, olive oil, safflower oil, sunflower oil, cotton oil, canola oil and the like. The most preferred lipid sources are safflower oil, canola oil and soybean oil.

Preferably, the lipid component of the composition of this. invention comprises, based on total caloric content of the composition, 4 to 10% of long chain fatty acids having about 14-24 carbon atoms and 0 to 20% of medium chain triglycerides having fatty acid chains of about 6-12 carbon atoms.

The lipid component preferably comprises omega-6 polyunsaturated fatty acids (i.e., linoleic acid) at 2-4% of total calories and omega-3 polyunsaturated fatty acids (i. e., **linolenic acid**) at 0.2-1.0% of total calories.

The level Of total lipid in the preferred composition is 4-30% (preferably about 6%) of calories which provides about 6.7 grams of lipid per liter. This level ensures adequate levels of essential fatty acids in the diet, particularly for those patients who receive smaller volumes (fewer calories) of formula. The use of canola or soybean oil allows for the addition of the **omega-3 fatty acid**, alpha-linoleic acid. This level will meet the nutrient needs of alpha-linolenic and linoleic acid and limit the possible negative effects of high fat formulas which include malabsorption, diarrhea and suppression of the immune system.

The composition of the invention comprises, based on total caloric content, from 65 to 80% carbohydrate component, preferably about 76%. Suitable carbohydrate sources for the. carbohydrate component can be those conventionally known and used in enteral feeding compositions. Sources of carbohydrate include, for example, cereals, vegetables, starches, glucoses, disaccharides, maltodextrins and the like. The preferred carbohydrates are maltodextrins and modified or hydrolyzed starches. The most preferred compositions of this invention in liquid form comprises about 190 grams of carbohydrate per liter.

The composition of the invention comprises, based on total caloric content, from 16 to 25% protein component, preferably about 18%. Suitable protein sources for the protein component can include conventional sources of intact protein, protein hydrolysates and crystalline amino acids used in enteral feeding compositions such as, for example, casein, soy, lactalbumin, egg albumen, whey and the like. The protein component of the claimed composition comprises based on free base 14 to 30% (preferably 22-23%) by weight glutamine and 5 to 33% (preferably 11-12%) by weight arginine. The glutamine and arginine can be in free base form, ingestible salt form, partially hydrolyzed protein form or intact protein form. Preferably, the composition comprises, based on total caloric content of the composition, arginine ranging from 1 to 6% based on the free base.

The constituents of protein are amino acids. All amino acids are obtained directly or indirectly from dietary protein or amino acids. Furthermore, the amino acids are utilized concomitantly for synthesis of tissue protein. No protein is stored in the body. There are two kinds of amino acids. Essential amino acids must be supplied by diet, where as nonessential amino acids can be produced by the body. Of the 22 identified amino acids, 9 are considered essential for infants and 8 have been designated essential for children and adults. Essential amino acids are not more important than non essential amino acids in the metabolic process. The distinction between the two protein groups is the necessity for dietary sources of essential amino acids. Other nutrients can be omitted from the diet of a well-nourished person and have little or no immediate effect on growth or appetite. Omission of a single amino acid from the diet or consumption of a diet with an imbalanced amino acid pattern results immediately in failure of the body to use all other amino acids except as an energy source. Dietary proteins vary greatly in amino acid composition.

Those proteins possessing an assortment of essential amino acids most nearly matching the body's protein requirements are considered to be of the highest biological value and will meet normal protein synthesis needs. Proteins derived from animal origin (especially eggs and milk) have the highest biological value, but vegetable protein foods can be combined in such a way that the overall amino acid composition of the mixture has a nutritional value comparable to that of "good animal protein". Proteins that have amino acid profiles with high biological value will meet normal protein syntheses needs. Radical alteration in amino acid intake, particularly if the concurrent energy intake is marginal, can result in a compromised environment for protein synthesis.
The proportion of essential amino acids in total protein should be at least about 40% in order to promote tissue restitution.

The composition of this invention has maintained its ratio of essential to nonessential amino acid even though arginine and glutamine have been added. This critical balance has been maintained through selected and careful calculations of each amino acid and has allowed for an amino acid profile which is considered to be of high biological value.

The amount and type of protein is vital to the critically ill patient. Attention should be paid to the amino acid composition, nitrogen needs as they relate to energy needs, and the metabolic changes of these patients. In general, protein requirements are elevated post-injury due to increased losses and greater needs for anabolism and tissue repair. Studies have shown that enteral fortification employing sufficient quantities of protein can accelerate the synthesis of visceral proteins and promote positive nitrogen balance and host defense factors.

Protein needs are tied to energy needs. The typical American diet reflects a nonprotein calorie (NPC) to grams of nitrogen (N) ratio of approximately 200 to 300:1, and the RDA is 0.8 gm/kg of body weight per day. The recovery from injury or illness is a dynamic process that varies among individuals. The metabolic response is a result of several factors, including the individuals' previous state of health, the extent of the injury, the type of surgical procedure required, and the type and extend of complications. Recovery also depends on the nutritional status of the individual. Because caloric expenditures and nitrogen excretion

are effected in parallel to stress, both the caloric intake and nitrogen content of the diet are examined together. Precise recommendations for protein allowance in critical care can vary and the present composition has NPC:N ranging from 150:1 to 80:1, depending on degree of protein depletion and severity of injury. The composition of this invention has increased its total nitrogen content (% of calories) and lowered its NPC:N. The nitrogen content of the composition can be calculated or measured using a variety of techniques such as described in Proteins--A Guide to Study by Physical and Chemical Methods, R. Haschemeyer, et al., John Wiley & Sons, p. 51, (1973) the disclosure of which is herein incorporated by reference.

Most enteral formulas use hydrolyzed cornstarch as the carbohydrate source. The degree of hydrolysis contributed to the formulas' osmolality, severeness and digestibility. The carbohydrate source in the preferred compositions of this invention comprise 96% hydrolyzed cornstarch and 4% modified starch. The preferred amount of carbohydrate is about 190 grams per liter providing about 76% of the total calories. The relatively lower carbohydrate source allows for increased levels of nitrogen and yet maintain a "moderate osmolality". The carbohydrate source is preferably free from lactose, (which may be a problem in the critically ill due to lactose intolerance) and preferably contains no sucrose, fructose or dietary fiber.

Arginine is included in the compositions of this invention although it is classified as a nonessential amino acid. It is not considered to be an essential dietary constituent for humans in the normal, unstressed human; the urea cycle provides sufficient arginine for maintenance. However, endogenous biosynthesis of arginine may not be sufficient for maximal tissue regeneration or positive nitrogen balance in trauma or stress. Dietary arginine enrichment may diminish protein catabolism and hence, reduce urinary nitrogen excretion in trauma or stress and improve immune function.

The level of arginine in the preferred composition is about 1-6% (more preferably about 2%) of calories providing about 5 grams per liter. The arginine level is based on experiments (for example, using a third degree 30% body surface area burn model in guinea pigs) which demonstrated that diets containing about 2% arginineo increased survival, improved delayed hypersensitivity as examined by dinitroflurobenzene, and heightened local bacterial containment as assessed by the size of the pustules after intradural staphylococcal injections. It has also been shown that plasma arginine concentrations had a high correlation with a number of parameters indicating resistance to infection, such as total protein, albumin, transferrin, C3 levels and opsonic index in severely burned children. Studies of surgical patients found that supplemental arginine significantly enhanced lymphocyte blastogeneses and increased CD4 phenotype (% T cells) postoperatively. The beneficial effect of arginine on the immune system appeared distinct from its more moderate effect on nitrogen balance.

Glutamine is utilized at a high rate by the intestinal cells in the basal state, while its uptake and metabolism increase even further in the course of catabolic illness. It has been proposed that glutamine deficiency may develop in the course of many catabolic diseases and that this deficiency may have an important impact on intestinal mucosal integrity and function. Increased uptake of glutamine by the gut in response to stress and critical illness spares glucose as an intestinal fuel.

Aside from glutamine's role in the gut, there is some evidence of other benefits: spares glucose as an intestinal fuel; supports release of gluconeogenic precursors; provides a respiratory fuel for fibroblasts and lymphoid tissue. Patients who are at high risk of developing glutamine "deficiency" may benefit from the incorporation of glutamine at 14-30% by weight of the protein component of the enteral nutritional composition of this invention.

Novel nutrients, such as carnitine, which under certain conditions may become essential, have been added to formulas. The daily requirement is unknown for mammalian species including humans. Carnitine is synthesized in the liver from the essential amino acids lysine and methionine. If the liver is impaired, it is possible that synthesis of carnitine may also be impaired. Since all of the long chain fatty acids supplied in the diet must be transported into the mitochondria via a carnitine pathway before they can be oxidized to produce energy, adequate levels of carnitine in the tissue are essential for this metabolic step. It has also been shown that carnitine improved the energy metabolism of patients receiving TPN support, and it improved muscle mass of hospitalized patients given supplemental carnitine. The typical carnitine intake of healthy adults on normal diets average 29-47 mg per day with a range of 0.18 to 319 mg per day. The preferred compositions of the invention comprise about 180 mg of carnitine or about 0.03% to about 0.05% by weight based on free base of carnitine per 1800 calories; said carnitine being in free base form or ingestible salt form.

Taurine, important for normal retinal development and in the synthesis of bile salts, may be essential for infants, children and perhaps critically ill adults. Some studies of patients with cystic fibrosis have shown improved fat absorption, growth, and weight gain following taurine supplementation of 30 mg/kg/day. Typical daily taurine intake is estimated to fall within a range of 9-372 mg per day. The preferred composition comprise about 360 mg taurine per 1800 calories or, based on free base, 0.07 to 0.09% by weight taurin; said taurine being in free base form or ingestible salt form.

In enteral nutrition support there is clearly a balance which must be maintained for: (1) the need to infuse nutrients into the patient and (2) the need to ensure that tolerance, absorption, and utilization of those nutrients are achieved. While it is recognized that meeting nutrient needs in lower volumes of formula is desirable, the nature of elemental formulas is such that concentrating nutrients inherently increases osmolar load. Hypertonic enteral formulas, especially when tube fed, may cause nausea, vomiting, cramping, abdominal distention and diarrhea in sensitive patients. The main determinants of the osmolality of a formula are simple carbohydrates, electrolytes and amino acids. Based on clinical experience the compromise of achieving a "moderate osmolality" and feeding appropriately 1800 to 2000 milliliters and 1800 to 2000 calories per day is acceptable for the compositions of the present invention.

The compositions of the invention can be in the form of a solid powder which is subsequently mixed with juices or other aqueous liquid or other flavoring agents. The solid powder form preferably has a caloric content from about 3 to about 4 calories per gram of the composition. The compositions can also be in the form of a ready-to-use aqueous liquid which preferably has a caloric content of about 1 calorie per milliliter. The aqueous compositions of the invention preferably have an

osmolality of about 630-690 mOsm per kilogram of water.

The enteral nutritional compositions of this invention may be administered via a nasogastric, nasointestinal, esophagostomy, gastrostomy, or jejunostomy feeding tube. Because of its homogeneity and low viscosity, small bore feeding tubes (16 gauge catheter or #5 French tube) may be used to optimize patient tolerance. The diet should be given at room temperature by continuous drip technique, or using a suitable infusion pump. At the 1 calorie per ml dilution, the composition supplies most of the daily fluid requirements. Additional fluids should be given when necessary to maintain hydration and adequate urine output.

The compositions can also be administered orally as a flavored drink served chilled over ice.

the invention based on solid A preferred composition of the invention based on solid weight is as follows:

INGREDIENT	AMOUNT (WT. %)
MALTODEXTRIN	69.32
L-GLUTAMINE	3.773
MODIFIED FOOD STARCH	3.773
L-LEUCINE	2.547
L-ARGININE ACETATE	2.536
SOYBEAN OIL	2.505
MAGNESIUM GLUCONATE	1.729
L-LYSINE ACETATE	1.486
L-VALINE	1.273
L-ISOLEUCINE	1.273
CALCIUM GLYCEROPHOSPHATE	1.258
L-PHENYLALANINE	1.078
L-METHIONINE	0.9265
CITRIC ACID	0.7755
L-THREONINE	0.7114
POTASSIUM CHLORIDE	0.5450
L-TYROSINE	0.4569
L-HISTIDINE MONOHYDROCHLORIDE	0.4528
SODIUM CITRATE	0.4402
L-ASPARTIC ACID	0.4192
L-PROLINE	0.3878
POTASSIUM CITRATE	0.3668
SODIUM PHOSPHATE DIBASIC	0.2851
L-TRYPTOPHAN	0.2587
L-SERINE	0.2159
CHOLINE BITARTRATE	0.2154
L-ALANINE	0.1937
GLYCINE	0.1886
POTASSIUM SORBATE	0.1467
POLYGLYCEROL ESTERS OF F.A.	0.1308
TAURINE	0.08300
VITAMIN E ACETATE	0.05659
ASCORBIC ACID	0.05072
L-CARNITINE	0.04150
BIOTIN	0.01761
ZINC SULFATE	0.01572
FERROUS SULFATE	0.01404
NIACINAMIDE	0.01199

VITAMIN A PALMITATE	0.01006
CALCIUM PANTOTHENATE	0.006749
CYANOCOBALAMIN	0.003668
COPPER GLUCONATE	0.003668
MANGANESE SULFATE	0.002369
FOLIC ACID	0.002348
VITAMIN K	0.002306
VITAMIN D	0.001761
PYRIDOXINE HYDROCHLORIDE	0.001467
POTASSIUM IODIDE	0.001149
RIBOFLAVIN	0.001048
THIAMIN HYDROCHLORIDE	0.000950
CHROMIC ACETATE	0.000179
SODIUM MOLYBDATE	0.000159
SODIUM SELENITE	0.000055

DETD The following examples are presented to demonstrate the present invention. The examples are intended to be illustrative and not limitative. The present invention includes the embodiments described and exemplified and equivalent embodiments.

EXAMPLE I

A composition within the scope of the present invention was prepared as follows:

A Fat Base composition was prepared having the following composition:

Ingredient	(Wt %)
maltodextrin	36.3000
modified starch	37.5000
soybean oil	24.9000
polyglycerol ester	1.3000

The Fat Base was prepared by:

1) Dissolving polyglycerol ester in warm deionized water;

2) Adding the solution from step 1 to a kettle containing 40 lbs. cold deionized water;

3) Adding maltodextrin and modified starch to the kettle, mixing well, and heating to 165.degree. F.;

4) When maltodextrin, modified starch and emulsifier blend reach 165.degree. F., add soybean oil and homogenize at 500 PSI second stage, 3000 PSI total.

5) Spray drying the product under the following conditions:

INLET 350.degree.-435.degree. F.

OUTLET 180.degree.-220.degree. F.

PRESSURE 1200.degree.-2200 PSI

PRODUCT TEMPERATURE 150.degree. F.

An Amino Acid Premix was prepared having the following composition:

Ingredient	Wt. %
L-glutamine	20.6609
L-leucine	13.9461
L-arginine acetate	13.8887
L-lysine acetate	8.1381
L-isoleucine	6.9731
L-valine	6.9731
L-phenylalanine	5.9033
L-methionine	5.0734
L-threonine	3.8957
L-tyrosine	2.5023
L-histidine HCL	2.4793
L-aspartic acid	2.2957
L-proline	2.1235
L-tryptophan	1.4164
L-serine	1.1823
L-alanine	1.0606
glycine	1.0330
taurine	0.4545

A Vitamin/Mineral Premix was prepared having the following composition:

Ingredient	Wt. %
magnesium gluconate	37.1184
calcium glycerophosphate	26.9952
potassium chloride	11.6979
sodium citrate	9.4483
trace mineral premix*	4.9576
potassium sorbate	3.1494
maltodextrin	2.2496
ascorbic acid	1.0888
vitamin E acetate	1.2148
biotin	0.3779
zinc sulfate	0.3374
ferrous sulfate	0.3014
niacinamide B3	0.2574
vitamin A palmitate	0.2160
calcium pantothenate	0.1449
cyanocobalamin B12	0.0787
copper gluconate	0.0787
manganese sulfate	0.0508
folic acid	0.0504
vitamin K	0.0495
vitamin D	0.0378
pridoxine hydrochloride	0.0315
potassium iodide	0.0247
riboflavin	0.0225
thiamin hydrochloride	0.0204

*The trace mineral premix comprised 99.8295% maldodextrin, 0.0776% chroni acetate, 0.0690% sodium molybdate and 0.0239% sodium selenite.

The following procedure was utilized in preparing the composition of the invention:

INGREDIENT	Wt. %
MALTODEXTRIN	65.33
AMINO ACID PREMIX	18.26
FAT BASE	10.06
VITAMIN/MINERAL PREMIX	4.659
CITRIC ACID	0.7756
POTASSIUM CITRATE	0.3668
SODIUM PHOSPHATE DIBASIC	0.2851
CHOLINE BITARTRATE	0.2154
L-CARNITINE	0.04150

Add 40.0 grams of maltodextrin to a mixer. Add 18.26 grams of the Amino. Acid Premix and 4.659 grams of the Vitamin/Mineral Premix to the mixer. Blend the following ingredients for ten minutes and add to the mixer:

MALTODEXTRIN	5.33 GMS
CHOLINE BITARTRATE	0.215 GMS
POTASSIUM CITRATE	0.367 GMS
CITRIC ACID	0.776 GMS
L-CARNITINE	0.042 GMS
SODIUM PHOSPHATE DIBASIC	0.285 GMS

Add 10.06 grams of the Fat Base and 20.00 grams of maltodextrin to the mixer and mix for 10 minutes.

CLM What is claimed is:

1. An enternal nutritional composition comprising, based on total caloric content of said composition, a) from 4% to 30% lipid component, b) from 65% to 80% carbohydrate component, and c) from 16% to 25% protein component, wherein said protein component consists of free amino acids in free base or ingestible salt form and comprises based on the free base 22% to 30% by weight glutamine and 11% to 33 by weight arginine, and at least 40% by weight essential amino acids, wherein said composition has a nonprotein calorie to grams of nitrogen ratio ranging from 150:1 to 80:1.

2. An enternal nutritional composition comprising, based on total caloric content of said composition, a) from 4% to 30% lipid component, b) from 65% to 80% carbohydrate component, and c) from 16% to 25% protein component, wherein said protein component consists of free amino acids in free base or ingestible salt form and comprises, based on the free base, 22% to 23% by weight glutamine and 11% to 12% by weight arginine and at least 40% by weight essential amino acids, wherein said composition has a nonprotein calorie to grams of nitrogen ratio raging from 150:1 to 80:1.

3. The composition of claims 1 or 2 wherein said lipid component comprises, based on total caloric content of said composition, 4% to 10% of 14-24-carbon long chain fatty acids and 0 to 20% of medium chain triglycerides having fatty acid chains Of 6-12 carbon atoms.

4. The composition of claims 1 or 2 comprising about 6% of said lipid component, about 76% of said carbohydrate component and about 18% of said protein component.

5. The composition of claims 1 or 2 wherein said composition has a nonprotein calorie to grams of nitrogen ratio of about 115:1.

6. The composition of claims 1 or 2 wherein said composition is in the form of a solid powder.

7. The composition of claim 6 wherein said composition has a caloric content from about 3 to about 4 calories per gram of said composition.

8. The composition of claims 1 or 2 wherein said composition is in the form of an aqueous liquid.

9. The composition of claim 8 wherein said composition has a caloric content of about 1 calorie per milliliter.

10. The composition of claim 8 wherein said composition has an osmolality of from 630 to 690 mOsm per kilogram of water.

11. The composition of claims 1 or 2 comprising based on the free base form from 0.03% to 0.04% by weight carnitine and about 0.07% to about 0.09% by weight taurine; said carnitine and taurine being in free base form or ingestible salt form.

12. An enteral nutritional composition comprising, based on total caloric content of said composition, a) from 4% to 30% lipid component, b) from 65% to 80% carbohydrate component, and c) from 16% to 25% protein component, said protein component comprising, in free base form,

ingestible salt form, partially hydrolyzed protein form or intact protein form, 14% to 30% by weight glutamine and 5% to 33% by weight arginine, based on the free base form, wherein said protein component contains an amino acid premix comprising about, based on total weight of

said amino acid premix:

Ingredient	Wt. %
L-glutamine	20.6609
L-leucine	13.9461
L-arginine acetate	13.8887
L-lysine acetate	8.1381
L-isoleucine	6.9731
L-valine	6.9731
L-phenylalanine	5.9033
L-methionine	5.0734
L-threonine	3.8957
L-tyrosine	2.5023
L-histidine HCl	2.4793
L-aspartic acid	2.2957
L-proline	2.1235
L-tryptophan	1.4164
L-serine	1.1823
L-alanine	1.0606
glycine	1.0330
taurine	0.4545:

wherein said composition has a nonprotein calorie to grams of nitrogen ranging from 150:1 to 80:1; and wherein said composition comprises by weight, in free base form or ingestible salt form, about 0,03% to 0.04% by weight carnitine and about 0.07 to 0.09% by weight taurine, based on the free base form of said carnitine and said taurine.

13. The composition of claim 12 wherein said composition is in the form of a solid powder.

14. The composition of claim 13 wherein said composition has a caloric content from about 3 to about 4 calories per gram of said composition.

15. The composition of claim 12 wherein said composition is in the form of an aqueous liquid.

16. The composition of claim 15 wherein said composition has a caloric content of about 1 calorie per milliliter.

17. The composition of claim 15 having an osmolality of from about 630-690 mOsm per kilogram of water.

INCL INCLM: 514/021.000
INCLS: 514/002.000; 424/439.000; 424/600.000; 424/679.000; 424/709.000; 426/062.000; 426/072.000; 426/073.000
NCL NCLM: 514/021.000
NCLS: 424/439.000; 424/600.000; 424/679.000; 424/709.000; 426/064.000; 426/072.000; 426/073.000; 514/002.000
IC [6]
ICM: A61K038-01
ICS: A61K033-14; A23L001-202; A23L001-30
EXF 514/2; 514/21; 514/773; 514/777; 424/439; 424/600; 424/709; 424/679; 426/64; 426/72; 426/73; 426/800; 426/801; 426/810
ARTU 181
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 19 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 96096350 EMBASE
DN 1996096350
TI Effects of modulation of **inflammatory** and immune parameters in patients with rheumatic and **inflammatory** disease receiving dietary supplementation of n-3 and n-6 fatty acids.
AU Kremer J.M.
CS Division of Rheumatology, Albany Medical College,Albany, NY 12208, United States
SO Lipids, (1996) 31/3 SUPPL. (S243-S247).
ISSN: 0024-4201 CODEN: LPDSAP
CY United States
DT Journal; Conference Article
FS 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
031 Arthritis and Rheumatism
037 Drug Literature Index
LA English
SL English
AB To describe the rationale and status of n-3 and n-6 fatty acid dietary supplementation in patients with **inflammatory** disease. The most recent literature is reviewed with a focus on rheumatoid arthritis (RA) as most investigations have described the use of n-3 supplements in this disease entity. Investigations from Europe, the United States, and Australia have described consistent improvement in tender joint scores with many investigators also observing improvements in morning stiffness. A meta analysis has confirmed the predictable improvement in tender joints. Recent studies also suggest that some patients with RA are able to discontinue nonsteroidal **antiinflammatory** drugs (NSAIDs) while receiving n 3 fatty acids. A large number of peer reviewed publications from around the world have established the utility of dietary supplementation with n-3 fatty acids in reducing tender joint counts and morning stiffness in patients with RA. Some patients are also able to discontinue NSAIDs while on these supplements.
CT Medical Descriptors:

***inflammation: DT, drug therapy**
***inflammation: TH, therapy**
*rheumatoid arthritis: DT, drug therapy
*rheumatoid arthritis: TH, therapy
clinical trial
conference paper
diet supplementation
enzyme activity
human
meta analysis
neutrophil
nonhuman
Drug Descriptors:
*diclofenac
*fish oil
***gamma linolenic acid**
*icosapentaenoic acid
*olive oil
***omega 3 fatty acid**
***omega 6 fatty acid**
*prostaglandin e1
arachidonate 5 lipoxygenase: EC, endogenous compound
arachidonic acid
bradykinin
cyclic amp: EC, endogenous compound
indometacin
interleukin 1beta
interleukin 2: EC, endogenous compound
leukotriene: EC, endogenous compound
leukotriene b4: EC, endogenous compound
leukotriene b5: EC, endogenous compound
linoleic acid
nonsteroid antiinflammatory agent: IT, drug interaction
nonsteroid antiinflammatory agent: DT, drug therapy
phospholipid
primrose oil
prostacyclin: EC, endogenous compound
prostaglandin: EC, endogenous compound
prostaglandin synthase: EC, endogenous compound
safflower oil
serotonin
thromboxane: EC, endogenous compound
unindexed drug
vegetable oil
RN (diclofenac) 15307-79-6, 15307-86-5; (fish oil) 8016-13-5; (gamma **linolenic acid**) 1686-12-0; (icosapentaenoic acid) 25378-27-2, 32839-30-8; (olive oil) 8001-25-0; (prostaglandin e1) 745-65-3; (arachidonate 5 lipoxygenase) 80619-02-9; (arachidonic acid) 506-32-1, 6610-25-9, 7771-44-0; (bradykinin) 58-82-2, 5979-11-3; (cyclic amp) 60-92-4; (indometacin) 53-86-1, 74252-25-8, 7681-54-1; (interleukin 2) 85898-30-2; (leukotriene b4) 71160-24-2; (leukotriene b5) 80445-66-5; (linoleic acid) 1509-85-9, 2197-37-7, 60-33-3, 822-17-3; (primrose oil) 65546-85-2; (prostacyclin) 35121-78-9, 61849-14-7; (prostaglandin synthase) 39391-18-9, 59763-19-8, 9055-65-6; (safflower oil) 8001-23-8; (serotonin) 50-67-9; (thromboxane) 66719-58-2

L13 ANSWER 20 OF 33 CAPLUS COPYRIGHT 2001 ACS
AN 1997:128695 CAPLUS
DN 126:143503
TI Dietary polyunsaturated fats and **inflammation**
AU James, M. J.; Cleland, L. G.
CS Rheumatology Unit, Royal Adelaide Hosp., Adelaide, 5000, Australia
SO Proc. Nutr. Soc. Aust. (**1996**), 20, 71-77
CODEN: PNSADB; ISSN: 0314-1004
PB Nutrition Society of Australia
DT Journal; General Review

LA English
CC 18-0 (Animal Nutrition)
AB A review with 20 refs. on the role of dietary n6 and n3 fats as determinants of **inflammation** and the possible cellular and biochem. mechanisms responsible. Dietary polyunsatd. fats are classified as n-3 or n-6 according to their double bond chem. and these chem. differences confer differential biol. effects on fatty acids from these two classes. In the modern Australian diet, the intake of n-6 fats exceeds that of n-3 fats by approx. 25-fold. This relative abundance of n-6 fat intake is reflected in the cell membranes where the ratio of n-6:n-3 PUFA is approx. 7:1. While this relative excess of n-6 to n-3 fat has been driven by agricultural and industrial changes as well as dietary changes aimed at lowering blood cholesterol levels, there is considerable evidence that increasing the amt. of dietary n-3 fat can suppress **inflammatory** mediator prodn. and can suppress **inflammation**. Animal studies using models of **inflammatory** disease have demonstrated that ingestion of fish oil, rich in n-3 fats, can suppress **inflammation**. In human studies, at least 11 double-blind, placebo-controlled clin. trials with rheumatoid arthritis patients have demonstrated that dietary supplements of fish oil can provide symptomatic benefits. The mechanisms for these clin. responses lie in the effects which n-3 fats have on the prodn. of **inflammatory** mediators. Dietary fish oil which contains 20- and 22-carbon n-3 fatty acids and **flaxseed oil** which contains their 18-carbon n-3 progenitor fatty acid, can inhibit the prodn. of the eicosanoid **inflammatory** mediators, prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) and the cytokine **inflammatory** mediators, interleukin-1.beta. (IL-1.beta.) and tumor necrosis factor-.alpha. (TNF.alpha.). Because n-6 fats can decrease the levels of n-3 fats in cell membranes, it is most likely that the optimum anti-**inflammatory** effects of n-3 fats will be within the context of diets also contg. lower levels of n-6 fats than those in the current Australian diet.
ST review
IT **Inflammation**
(dietary polyunsatd. fats and **inflammation**)
IT **Omega-3 fatty acids**
Omega-6 fatty acids
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(dietary polyunsatd. fats and **inflammation**)

L13 ANSWER 21 OF 33 CAPLUS COPYRIGHT 2001 ACS
AN 1995:943658 CAPLUS
DN 123:338084
TI Pet food product containing omega-6 and **omega-3 fatty acids**.
IN Reinhart, Gregory A.
PA Iams Co., USA
SO Eur. Pat. Appl., 14 pp.
CODEN: EPXXDW
DT Patent
LA English
IC ICM A23K001-16
ICS A23K001-18; A23K001-10
CC 17-12 (Food and Feed Chemistry)
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 678247	A1	19951025	EP 1995-302540	19950418 <--
	R: BE, DE, FR, GB				
	CA 2147109	AA	19951019	CA 1995-2147109	19950413 <--
	AU 9516520	A1	19951026	AU 1995-16520	19950418 <--
	JP 08038063	A2	19960213	JP 1995-92576	19950418 <--
PRAI	US 1994-228669		19940418		

AB A pet food product is provided for reducing **inflammatory** and allergic skin responses. The pet food contains .omega.-6- and .**omega.-3-fatty acids** in the ratio 3:1 to 10:1. Also, .gtoreq.3% the total fatty acids in the pet food compn. are .**omega.-6-fatty acids**.

ST pet food

IT Horse
(feed contg. omega-6 and **omega-3 fatty acids** for)

IT Canis familiaris
Felis catus
(food contg. omega-6 and **omega-3 fatty acids** for)

IT Linseed oil
RL: FFD (Food or feed use); BIOL (Biological study); USES (Uses)
(pet food contg.)

IT Food
(pet; contg. omega-6 and **omega-3 fatty acids**)

IT Fats and Glyceridic oils
RL: FFD (Food or feed use); BIOL (Biological study); USES (Uses)
(fish, pet food contg.)

IT Fatty acids, biological studies
RL: FFD (Food or feed use); BIOL (Biological study); USES (Uses)
(polyunsatd., n-3, pet food contg.)

IT Fatty acids, biological studies
RL: FFD (Food or feed use); BIOL (Biological study); USES (Uses)
(polyunsatd., n-6, pet food contg.)

IT 463-40-1, .alpha.-**Linolenic acid** 6217-54-5, Docosahexaenoic acid 10417-94-4, Eicosapentaenoic acid
RL: FFD (Food or feed use); BIOL (Biological study); USES (Uses)
(pet food contg.)

L13 ANSWER 22 OF 33 USPATFULL

AN 95:69268 USPATFULL

TI Enteral nutritional composition having balanced amino acid profile

IN Schmidl, Mary K., Arden Hills, MN, United States
Kvamme, Candis, Brooklyn Park, MN, United States

PA Sandoz Nutrition Ltd., Berne, Switzerland (non-U.S. corporation)

PI US 5438042 19950801 <--

AI US 1993-134226 19931008 (8)

DT Utility

FS Granted

REP

US 3697287	Oct 1972	426/073.000	Winitz
US 3698912	Oct 1972	426/656.000	Winitz
US 3701666	Oct 1972	426/311.000	Winitz
US 4414238	Nov 1983	426/602.000	Schmidt
US 4670268	Jun 1987	426/072.000	Mahmond
US 4737364	Apr 1988	424/195.100	Kalogris
US 4752618	Jun 1988	514/549.000	Mascioli et al.
US 4847296	Jul 1989	514/552.000	Babayan et al.
US 4921877	May 1990	514/866.000	Cashmere et al.
US 5053387	Oct 1991	514/002.000	Alexander
US 5221668	Jun 1993	514/023.000	Henningfield et al.
US 5231085	Jul 1993	514/044.000	Alexander et al.

REN J. W. Alexander et al, Ann. Surg., vol. 192, pp. 505-517 (1980).
L. Dominioni et al, J. Burn Care Rehab., vol. 5, pp. 106-112 (1984).
Bower et al, Ann. Surg., vol. 203, pp. 13-20, (1986).
Cerra et al, Ann. Surg., vol. 192, pp. 570-580 (1980).
Cerra et al, Surgery, vol. 92, pp. 192-198 (1982).
Cerra et al, Surgery, vol. 98, pp. 632-638 (1985).
D. Law et al, Ann. Surg., vol. 179, pp. 168-173 (1974).
L. Dominioni et al, Surg. Forum, vol. 34, pp. 99-101 (1983).
Food and Nutrition Board-National Research Council, Recommended Dietary Allowances, 10th Ed, Nat. Acad. of Sciences, pp. 66-67 (1989).
M. Donnelly et al, J. Cell Physiol. vol. 89, pp. 39-52 (1976).

EXNAM Primary Examiner: Wityshyn, Michael G.; Assistant Examiner: Degen, Nancy
LREP Honor, Robert S., Battle, Carl W.
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN No Drawings
AB An enteral nutritional composition comprising 4-30% of a lipid component, 65-80% of a carbohydrate component, and 16-25% of a protein component, based on total caloric content is disclosed. This protein component of this composition comprises, by weight, 14-30% glutamine and 5-33% arginine. The non-protein calorie to grams of nitrogen ratio ranges from 150:1 and 80:1.

SUMM BACKGROUND OF THE INVENTION INFORMATION DISCLOSURE

In general, enteral nutrition compositions may be administered orally or by tube feeding. Numerous enteral formulations are utilized in patients with a hypermetabolic state as effected by burns, trauma, surgery and in patients with malnutrition, chronic illness and in disorders resulting from prolonged periods of reduced oral intake resulting from cerebral vascular accidents or a comatose state.

The enteral compositions have provided benefits and advantages to parenteral nutrition. Elemental diets are often indicated for patients who have a reduced gastro-intestinal absorptive surface or exocrine pancreatic insufficiencies. A few of the clinical indications for elemental formulas include: pancreatitis, short gut syndrome, radiation enteritis, GI cutaneous fistulas and Crohn's disease. For some patients, they may also be useful as a transition feeding or even replace total parenteral nutrition (TPN). This recommendation is based on recent clinical findings that demonstrate elemental diets when compared to TPN result in fewer complications, reduced patient length of stay in the ICU and are less expensive. Elemental diets are composed of low molecular weight nutrients and require minimal digestive and absorptive capability. The protein source consists of free amino acids and contains essential and non-essential amino acids. Carbohydrate is typically composed of glucose and hydrolyzed cornstarch (maltodextrin) while the fat content is usually low and primarily consists of essential fatty acids. These diets have minimal residue because of the efficient absorption of the nutrients provided in an elemental form. Most practitioners find that initiating feeding at full strength using low delivery rates is well tolerated, even though elemental formulas are, by nature, hyperosmolar (greater than 300 mOsm/Kg H.sub.2 O). However, in selected cases, initiating feeding with a dilute formula may be preferred. Elemental diets are often administered by needle catheter jejunostomy or endoscopically placed percutaneous jejunal tubes (PEJ) or nasoenteric small bowel feeding tubes in the critically ill patient.

ISOCAL is an enteral formulation by Mead Johnson which utilizes casein and soy for its protein source, glucose oligosacchrides for its carbohydrate source and soy oil and medium chain triglycerides (MCT) oil for its lipid source.

OSMOLITE is manufactured by Ross and utilizes as its protein source casein and soy, corn starch for its carbohydrate source and fifty percent MCT oil, forty percent corn oil and ten percent soy oil for its lipid source.

ENSURE is manufactured by Ross and utilizes casein and soy for protein source, corn starch and sucrose for a carbohydrate source and corn oil for a lipid source.

SUSTACAL manufactured by Mead Johnson utilizes casein and soy for its protein source, corn syrup and sucrose for its carbohydrate source and soy oil for its lipid source.

ENSURE PLUS manufactured by Ross is a high protein, high calorie composition using soy and casein for its protein source, corn starch and glucose for its carbohydrate source and corn oil for its lipid source.

MAGNACAL manufactured by Sherwood Medical is a high density composition with 2.0 calories/ml. MAGNACAL utilizes casein for its protein source, corn syrup for its carbohydrate source and soy oil for its lipid source.

TRAUMACAL manufactured by Mead Johnson utilizes casein for its protein source, corn syrup and sucrose for its carbohydrate source and 70 percent soy bean oil and 30 percent MCT oil for its lipid source.

ISOTEIN HN is manufactured by Sandoz and utilizes lactalbumin for its protein source, maltodextrin for its carbohydrate source and soy oil and MCT oil for its lipid source.

VIVONEX T.E.N. is manufactured by Sandoz and comprises branched chain amino acids, glutamine and arginine as the protein source, safflower oil as the lipid source, and maltodextrin and modified starch as the carbohydrate source.

IMPACT is manufactured by Sandoz and comprises arginine and caseinates as the protein source, maltodextrins as the carbohydrate, and menhaden oil and structured lipids as the lipids source.

U.S. Pat. No. 4,752,618 describes a dietary supplement and method of minimizing infections therewith, comprising omega-3 and **omega-6 fatty acid** such as safflower oil and menhaden oil.

U.S. Pat. No. 4,847,296 describes triglyceride preparations for enteral administration to prevent catabolism and increase protein synthesis in subjects undergoing severe metabolic stress.

U.S. Pat. No. 5,053,387 describes enteral compositions for treating traumatic injury comprising an intact protein (from lactalbumin egg albumen or whey and the like), arginine, carbohydrate (glucose polymers, disaccharides, starches and the like), lipid comprising **omega-3 fatty acids** of fish oil, and necessary vitamins and minerals.

U.S. Pat. No. 5,231,085 describes enteral compositions comprising arginine, ornithine, a nucleobase, omega-3 polyunsaturated fatty acids, and omega-6 polyunsaturated fatty acids.

Other background references on enteral feeding compositions and methods include:

Alexander, J. W., MacMillan, B. G., Stinnett. J. P. et al: Beneficial effects of aggressive protein feeding in severely burned children. Ann. Surg. 192:505-517, 1980;

Dominioni, L., Trocki, O., Mochizuke, H., et al: Prevention of severe postburn hypermetabolism and catabolism by immediate intragastric feeding. J. Burn Care Rehab. 5:106-112, 1984;

Bower, R. H., Muggia-Sullam, M., Vallgren, S., et al: Branched chain amino acid entriched solutions in the septic patient. A randomized. prospective trial. Ann. Surg. 203:13-20, 1986;

Cerra, F. G., Siegal, J. H., Coleman, B., et al: Septic autocannibalism:
A failure of exogenous nutritional support. Ann. Surg. 192:570-580, 1980;

Cerra, F. B., Upson, D., Angelico, R., et al: Branched chains support post-operative protein synthesis. Surgery 92:192-198, 1982;

Cerra, F. B. Shronts, E. P., Konstantinides, N. N., et al: Enteral feeding in sepsis: A prospective randomized, double-blind trial. Surgery
98:632-638, 1985;

Law, D. K., Durdick, S. J. and abdon, N. I.: The effect of dietary protein depletion on immuno competence: the importance of nutritional repletion prior to immunologic induction. Ann. Surg. 179:168-173, 1974;

Dominioni, L., Trocki, O., Fang, C. H., and Alexander, J. W.: Nitrogen balance and liver changes in burned guinea pigs under going prolonged high protein enteral feeding. Surg. Forum 34:99-101, 1983;

Food and Nutrition Board, National Research Council, Recommended Dietary
Allowances, 10th edition. Washington, D.C., National Academy of Sciences, 1989; and

Donnelly, M. and Scheffler, I. E.: Energy metabolism in respiratory deficient and mild type Chinese hamster fibroblasts in culture. J. Cell Physical. 89:39-51, 1976.

Improvements to the enteral feeding compositions are continually sought to provide greater patient benefits. It is the object of the present invention to provide an improved enteral nutritional composition that is
nutritionally balanced and which provide complete nutritional support for critically ill patients.

SUMMARY OF THE INVENTION

The present invention relates to an enteral nutritional composition comprising based on total caloric content of said composition,

a) from 4 to 30% lipid component,

b) from 65 to 80% carbohydrate component, and

c) from 16 to 25% protein component, said protein component comprising based on the free base 14 to 30% by weight glutamine and 5 to 33% by weight arginine, said glutamine and arginine being in free base form, ingestible salt form, partially hydrolyzed protein form or intact protein form,

wherein said composition has a nonprotein calorie to grams of nitrogen ratio ranging from 150:1 to 80:1.

The composition can be in the form of a solid powder (which is subsequently mixed with water or other suitable liquid carriers) or in the form of a ready-to-use homogenous aqueous liquid. The composition is

useful in the dietary management of stress, trauma, burns, malnutrition, sepsis, **inflammatory** bowel disease, cancer, intestinal atresia, pancreatitis, fistula, short-gut syndrome, acquired immunodeficiency syndrome, cachexia, and other stressed and catabolic conditions.

It is a further object of this invention to provide an improved enteral feeding composition having higher total nitrogen, lower ratio of nonprotein calorie to gram of nitrogen ratio and improved amino acid profile. It is another object of this invention to provide improved enteral feeding composition containing carnitine and taurine and the resultant benefits therefrom. It is an even further object of this invention to provide a method of treating stressed catabolic patients by enteral administration of the improved feeding compositions of this invention.

DETAILED DESCRIPTION

This invention relates to improved enteral nutritional compositions which are useful in treating stressed and catabolic conditions. The enteral nutritional composition of this invention comprises, based on total caloric content of said composition,

a) from 4 to 30% lipid component,

b) from 65 to 80% carbohydrate component, and

c) from 16 to 25% protein component, said protein component comprising based on the free base 14 to 30% by weight glutamine and 5 to 33% by weight arginine, said glutamine and arginine being in free base form, ingestible salt form, partially hydrolyzed protein form or intact protein form,

wherein said composition has a nonprotein calorie to grams of nitrogen ratio ranging from 150:1 to 80:1.

The composition comprises based on total calories from 4-30% (preferably about 6%) lipid component. Adequate fat intake is important as a source of energy, essential fatty acids and carrier of fat soluble vitamins. Relatively low levels of fat (i.e., <3% calories) may be inadequate on a long-term basis (i.e., >2 weeks) to prevent essential fatty acid deficiency. Suitable lipids for use in the present invention include, any of the conventional saturated and unsaturated fatty acids, glycerides and other nutritionally acceptable fat sources known in the art, such as animal oils, fish oils, vegetable oils and synthetic lipids. Lipid sources include, for example, medium chain triglycerides, corn oil, soybean oil, peanut oil, olive oil, safflower oil, sunflower oil, cotton oil, canola oil and the like. The most preferred lipid sources are safflower oil, canola oil and soybean oil.

Preferably, the lipid component of the composition of this invention comprises, based on total caloric content of the composition, 4 to 10% of long chain fatty acids having about 14-24 carbon atoms and 0 to 20% of medium chain triglycerides having fatty acid chains of about 6-12 carbon atoms.

The lipid component preferably comprises omega-6 polyunsaturated fatty acids (i.e., linoleic acid) at 2-4% of total calories and omega-3 polyunsaturated fatty acids (i.e., **linolenic acid**) at 0.2-1.0% of total calories.

The level of total lipid in the preferred composition is 4-30%

(preferably about 6%) of calories which provides about 6.7 grams of lipid per liter. This level ensures adequate levels of essential fatty acids in the diet, particularly for those patients who receive smaller volumes (fewer calories) of formula. The use of canola or soybean oil allows for the addition of the **omega-3 fatty acid**, alpha-linoleic acid. This level will meet the nutrient needs of alpha-linolenic and linoleic acid and limit the possible negative effects of high fat formulas which include malabsorption, diarrhea and suppression of the immune system.

The composition of the invention comprises, based on total caloric content, from 65 to 80% carbohydrate component, preferably about 76%. Suitable carbohydrate sources for the carbohydrate component can be those conventionally known and used in enteral feeding compositions. Sources of carbohydrate include, for example, cereals, vegetables, starches, glucoses, disaccharides, maltodextrins and the like. The preferred carbohydrates are maltodextrins and modified or hydrolyzed starches. The most preferred compositions of this invention in liquid form comprises about 190 grams of carbohydrate per liter.

The composition of the invention comprises, based on total caloric content, from 16 to 25% protein component, preferably about 18%. Suitable protein sources for the protein component can include conventional sources of intact protein, protein hydrolysates and crystalline amino acids used in enteral feeding compositions such as, for example, casein, soy, lactalbumin, egg albumen, whey and the like. The protein component of the claimed composition comprises based on free base 14 to 30% (preferably 22-23%) by weight glutamine and 5 to 33% (preferably 11-12%) by weight arginine. The glutamine and arginine can be in free base form, ingestible salt form, partially hydrolyzed protein form or intact protein form. Preferably, the composition comprises, based on total caloric content of the composition, arginine ranging from 1 to 6% based on the free base.

The constituents of protein are amino acids. All amino acids are obtained directly or indirectly from dietary protein or amino acids. Furthermore, the amino acids are utilized concomitantly for synthesis of tissue protein. No protein is stored in the body. There are two kinds of amino acids. Essential amino acids must be supplied by diet, where as nonessential amino acids can be produced by the body. Of the 22 identified amino acids, 9 are considered essential for infants and 8 have been designated essential for children and adults. Essential amino acids are not more important than non essential amino acids in the metabolic process. The distinction between the two protein groups is the necessity for dietary sources of essential amino acids. Other nutrients can be omitted from the diet of a well-nourished person and have little or no immediate effect on growth or appetite. Omission of a single amino acid from the diet or consumption of a diet with an imbalanced amino acid pattern results immediately in failure of the body to use all other amino acids except as an energy source. Dietary proteins vary greatly in amino acid composition.

Those proteins possessing an assortment of essential amino acids most nearly matching the body's protein requirements are considered to be of the highest biological value and will meet normal protein synthesis needs. Proteins derived from animal origin (especially eggs and milk) have the highest biological value, but vegetable protein foods can be

combined in such a way that the overall amino acid composition of the mixture has a nutritional value comparable to that of "good animal protein". Proteins that have amino acid profiles with high biological value will meet normal protein syntheses needs. Radical alteration in amino acid intake, particularly if the concurrent energy intake is marginal, can result in a compromised environment for protein synthesis.

The proportion of essential amino acids in total protein should be at least about 40% in order to promote tissue restitution.

The composition of this invention has maintained its ratio of essential to nonessential amino acid even though arginine and glutamine have been added. This critical balance has been maintained through selected and careful calculations of each amino acid and has allowed for an amino acid profile which is considered to be of high biological value.

The amount and type of protein is vital to the critically ill patient. Attention should be paid to the amino acid composition, nitrogen needs as they relate to energy needs, and the metabolic changes of these patients. In general, protein requirements are elevated post-injury due to increased losses and greater needs for anabolism and tissue repair. Studies have shown that enteral fortification employing sufficient quantities of protein can accelerate the synthesis of visceral proteins and promote positive nitrogen balance and host defense factors.

Protein needs are tied to energy needs. The typical American diet reflects a nonprotein calorie (NPC) to grams of nitrogen (N) ratio of approximately 200 to 300:1, and the RDA is 0.8 gm/kg of body weight per day. The recovery from injury or illness is a dynamic process that varies among individuals. The metabolic response is a result of several factors, including the individuals previous state of health, the extent of the injury, the type of surgical procedure required, and the type and extend of complications. Recovery also depends on the nutritional status of the individual. Because caloric expenditures and nitrogen excretion are effected in parallel to stress, both the caloric intake and nitrogen content of the diet are examined together. Precise recommendations for protein allowance in critical care can vary and the present composition has NPC:N ranging from 150:1 to 80:1, depending on degree of protein depletion and severity of injury. The composition of this invention has increased its total nitrogen content (% of calories) and lowered its NPC:N. The nitrogen content of the composition can be calculated or measured using a variety of techniques such as described in Proteins--A Guide to Study by Physical And Chemical Methods, R. Haschemeyer, et al., John Wiley & Sons, p. 51, (1973) the disclosure of which is herein incorporated by reference.

Most enteral formulas use hydrolyzed cornstarch as the carbohydrate source. The degree of hydrolysis contributed to the formulas' osmolality, severeness and digestibility. The carbohydrate source in the preferred compositions of this invention comprise 96% hydrolyzed cornstarch and 4% modified starch. The preferred amount of carbohydrate is about 190 grams per liter providing about 76% of the total calories. The relatively lower carbohydrate source allows for increased levels of nitrogen and yet maintain a "moderate osmolality". The carbohydrate source is preferably free from lactose, (which may be a problem in the critically ill due to lactose intolerance) and preferably contains no sucrose, fructose or dietary fiber.

Arginine is included in the compositions of this invention although it is classified as a nonessential amino acid. It is not considered to be an essential dietary constituent for humans in the normal, unstressed

human; the urea cycle provides sufficient arginine for maintenance. However, endogenous biosynthesis of arginine may not be sufficient for maximal tissue regeneration or positive nitrogen balance in trauma or stress. Dietary arginine enrichment may diminish protein catabolism and hence, reduce urinary nitrogen excretion in trauma or stress and improve immune function.

The level of arginine in the preferred composition is about 1-6% (more preferably about 2%) of calories providing about 5 grams per liter. The arginine level is based on experiments (for example, using a third degree 30% body surface area burn model in guinea pigs) which demonstrated that diets containing about 2% arginine increased survival, improved delayed hypersensitivity as examined by dinitroflurobenzene, and heightened local bacterial containment as assessed by the size of the pustules after intradural staphylococcal injections. It has also been shown that plasma arginine concentrations had a high correlation with a number of parameters indicating resistance to infection, such as total protein, albumin, transferrin, C3 levels and opsonic index in severely burned children. Studies of surgical patients found that supplemental arginine significantly enhanced lymphocyte blastogeneses and increased CD4 phenotype (% T cells) postoperatively. The beneficial effect of arginine on the immune system appeared distinct from its more moderate effect on nitrogen balance.

Glutamine is utilized at a high rate by the intestinal cells in the basal state, while its uptake and metabolism increase even further in the course of catabolic illness. It has been proposed that glutamine deficiency may develop in the course of many catabolic diseases and that this deficiency may have an important impact on intestinal mucosal integrity and function. Increased uptake of glutamine by the gut in response to stress and critical illness spares glucose as an intestinal fuel.

Aside from glutamine's role in the gut, there is some evidence of other benefits: spares glucose as an intestinal fuel; supports release of gluconeogenic precursors; provides a respiratory fuel for fibroblasts and lymphoid tissue. Patients who are at high risk of developing glutamine "deficiency" may benefit from the incorporation of glutamine at 14-30% by weight of the protein component of the enteral nutritional composition of this invention.

Novel nutrients, such as carnitine, which under certain conditions may become essential, have been added to formulas. The daily requirement is unknown for mammalian species including humans. Carnitine is synthesized in the liver from the essential amino acids lysine and methionine. If the liver is impaired, it is possible that synthesis of carnitine may also be impaired. Since all of the long chain fatty acids supplied in the diet must be transported into the mitochondria via a carnitine pathway before they can be oxidized to produce energy, adequate levels of carnitine in the tissue are essential for this metabolic step. It has also been shown that carnitine improved the energy metabolism of patients receiving TPN support, and it improved muscle mass of hospitalized patients given supplemental carnitine. The typical carnitine intake of healthy adults on normal diets average 29-47 mg per day with a range of 0.18 to 319 mg per day. The preferred compositions of the invention comprise about 180 mg of carnitine or about 0.03% to about 0.05% by weight based on free base of carnitine per 1800 calories; said carnitine being in free base form or ingestible salt form.

Taurine, important for normal retinal development and in the synthesis

of bile salts, may be essential for infants, children and perhaps critically ill adults. Some studies of patients with cystic fibrosis have shown improved fat absorption, growth, and weight gain following taurine supplementation of 30 mg/kg/day. Typical daily taurine intake is estimated to fall within a range of 9-372 mg per day. The preferred composition comprise about 360 mg taurine per 1800 calories or, based on free base, 0.07 to 0.09% by weight taurin; said taurine being in free base form or ingestible salt form.

In enteral nutrition support there is clearly a balance which must be maintained for: (1) the need to infuse nutrients into the patient and (2) the need to ensure that tolerance, absorption, and utilization of those nutrients are achieved. While it is recognized that meeting nutrient needs in lower volumes of formula is desirable, the nature of elemental formulas is such that concentrating nutrients inherently increases osmolar load. Hypertonic enteral formulas, especially when tube fed, may cause nausea, vomiting, cramping, abdominal distention and diarrhea in sensitive patients. The main determinants of the osmolality of a formula are simple carbohydrates, electrolytes and amino acids. Based on clinical experience the compromise of achieving a "moderate osmolality" and feeding appropriately 1800 to 2000 milliliters and 1800 to 2000 calories per day is acceptable for the compositions of the present invention.

The compositions of the invention can be in the form of a solid powder which is subsequently mixed with juices or other aqueous liquid or other flavoring agents. The solid powder form preferably has a caloric content from about 3 to about 4 calories per gram of the composition. The compositions can also be in the form of a ready-to-use aqueous liquid which preferably has a caloric content of about 1 calorie per milliliter. The aqueous compositions of the invention preferably have an osmolality of about 630-690 mOsm per kilogram of water.

The enteral nutritional compositions of this invention may be administered via a nasogastric, nasointestinal, esophagostomy, gastrostomy, or jejunostomy feeding tube. Because of its homogeneity and low viscosity, small bore feeding tubes (16 gauge catheter or #5 French tube) may be used to optimize patient tolerance. The diet should be given at room temperature by continuous drip technique, or using a suitable infusion pump. At the 1 calorie per ml dilution, the composition supplies most of the daily fluid requirements. Additional fluids should be given when necessary to maintain hydration and adequate urine output.

The compositions can also be administered orally as a flavored drink served chilled over ice. A preferred composition of the invention based on solid weight is as follows:

INGREDIENT	AMOUNT (WT. %)
MALTODEXTRIN	69.32
L-GLUTAMINE	3.773
MODIFIED FOOD STARCH	3.773
L-LEUCINE	2.547
L-ARGININE ACETATE	2.536
SOYBEAN OIL	2.505
MAGNESIUM GLUCONATE	1.729

L-LYSINE ACETATE	1.486
L-VALINE	1.273
L-ISOLEUCINE	1.273
CALCIUM GLYCEROPHOSPHATE	1.258
L-PHENYLALANINE	1.078
L-METHIONINE	0.9265
CITRIC ACID	0.7755
L-THREONINE	0.7114
POTASSIUM CHLORIDE	0.5450
L-TYROSINE	0.4569
L-HISTIDINE MONOHYDROCHLORIDE	0.4528
SODIUM CITRATE	0.4402
L-ASPARTIC ACID	0.4192
L-PROLINE	0.3878
POTASSIUM CITRATE	0.3668
SODIUM PHOSPHATE DIBASIC	0.2851
L-TRYPTOPHAN	0.2587
L-SERINE	0.2159
CHOLINE BITARTRATE	0.2154
L-ALANINE	0.1937
GLYCINE	0.1886
POTASSIUM SORBATE	0.1467
POLYGLYCEROL ESTERS OF F.A.	0.1308
TAURINE	0.08300
VITAMIN E ACETATE	0.05659
ASCORBIC ACID	0.05072
L-CARNITINE	0.04150
BIOTIN	0.01761
ZINC SULFATE	0.01572
FERROUS SULFATE	0.01404
NIACINAMIDE	0.01199
VITAMIN A PALMITATE	0.01006
CALCIUM PANTOTHENATE	0.006749
CYANOCOBALAMIN	0.003668
COPPER GLUCONATE	0.003668
MANGANESE SULFATE	0.002369
FOLIC ACID	0.002348
VITAMIN K	0.002306
VITAMIN D	0.001761
PYRIDOXINE HYDROCHLORIDE	0.001467
POTASSIUM IODIDE	0.001149
RIBOFLAVIN	0.001048
THIAMIN HYDROCHLORIDE	0.000950
CHROMIC ACETATE	0.000179
SODIUM MOLYBDATE	0.000159
SODIUM SELENITE	0.000055

DETD The following examples are presented to demonstrate the present invention. The examples are intended to be illustrative and not limitative. The present invention includes the embodiments described and exemplified and equivalent embodiments.

EXAMPLE I

A composition within the scope of the present invention was prepared as follows:

A Fat Base composition was prepared having the following composition:

Ingredient	(Wt %)
maltodextrin	36.3000
modified starch	37.5000
soybean oil	24.9000
polyglycerol ester	1.3000

The Fat Base was prepared by:

1) Dissolving polyglycerol ester in warm deionized water;

2) Adding the solution from step 1 to a kettle containing 40 lbs. cold deionized water;

3) Adding maltodextrin and modified starch to the kettle, mixing well, and heating to 165.degree. F.;

4) When maltodextrin, modified starch and emulsifier blend reach 165.degree. F., add soybean oil and homogenize at 500 PSI second stage, 3000 PSI total.

5) Spray drying the product under the following conditions:

INLET 350.degree.-435.degree. F.

OUTLET 180.degree.-220.degree. F.

PRESSURE 1200.degree.-2200 PSI

PRODUCT TEMPERATURE 150.degree. F.

An Amino Acid Premix was prepared having the following composition:

Ingredient	Wt. %
L-glutamine	20.6609
L-leucine	13.9461
L-arginine acetate	13.8887
L-lysine acetate	8.1381
L-isoleucine	6.9731
L-valine	6.9731
L-phenylalanine	5.9033
L-methionine	5.0734
L-threonine	3.8957
L-tyrosine	2.5023
L-histidine HCL	2.4793
L-aspartic acid	2.2957
L-proline	2.1235
L-tryptophan	1.4164
L-serine	1.1823
L-alanine	1.0606
glycine	1.0330
taurine	0.4545

A Vitamin/Mineral Premix was prepared having the following composition:

Ingredient	Wt. %

magnesium gluconate	37.1184
calcium glycerophosphate	26.9952
potassium chloride	11.6979
sodium citrate	9.4483
trace mineral premix*	4.9576
potassium sorbate	3.1494
maltodextrin	2.2496
ascorbic acid	1.0888
vitamin E acetate	1.2148
biotin	0.3779
zinc sulfate	0.3374
ferrous sulfate	0.3014
niacinamide B3	0.2574
vitamin A palmitate	0.2160
calcium pantothenate	0.1449
cyanocobalamin B12	0.0787
copper gluconate	0.0787
manganese sulfate	0.0508
folic acid	0.0504
vitamin K	0.0495
vitamin D	0.0378
pridoxine hydrochloride	0.0315
potassium iodide	0.0247
riboflavin	0.0225
thiamin hydrochloride	0.0204

*The trace mineral premix comprised 99.8295% maldodextrin, 0.0776% chroni acetate, 0.0690% sodium molybdate and 0.0239% sodium selenite.

The following procedure was utilized in preparing the composition of the invention:

INGREDIENT	Wt. %
MALTODEXTRIN	65.33
AMINO ACID PREMIX	18.26
FAT BASE	10.06
VITAMIN/MINERAL PREMIX	4.659
CITRIC ACID	0.7756
POTASSIUM CITRATE	0.3668
SODIUM PHOSPHATE DIBASIC	0.2851
CHOLINE BITARTRATE	0.2154
L-CARNITINE	0.04150

Add 40.0 grams of maltodextrin to a mixer. Add 18.26 grams of the Amino Acid Premix and 4.659 grams of the Vitamin/Mineral Premix to the mixer. Blend the following ingredients for ten minutes and add to the mixer:

MALTODEXTRIN	5.33 GMS
CHOLINE BITARTRATE	0.215 GMS
POTASSIUM CITRATE	0.367 GMS
CITRIC ACID	0.776 GMS
L-CARNITINE	0.042 GMS

SODIUM PHOSPHATE DIBASIC 0.285 GMS

Add 10.06 grams of the Fat Base and 20.00 grams of maltodextrin to the mixer and mix for 10 minutes.

CLM What is claimed is:

1. An enteral nutritional composition comprising, based on total caloric content of said composition, a) from 4% to 30% lipid component, b) from 65% to 80% carbohydrate component, and c) from 16% to 25% protein component, wherein said composition has a nonprotein calorie to grams of nitrogen ratio ranging from 150:1 to 80:1, and has the following formulation by solid weight:

Ingredient	Wt %
maltodextrin	69.32
L-glutamine	3.773
modified food starch	3.773
L-leucine	2.547
L-arginine acetate	2.536
soybean oil	2.505
magnesium gluconate	1.729
L-lysine acetate	1.486
L-valine	2.374
L-isoleucine	1.273
calcium glycerophosphate	1.258
L-phenylalanine	1.078
L-methionine	0.9265
citric acid	0.7755
L-threonine	0.7114
potassium chloride	0.5450
L-tyrosine	0.4569
L-histidine monohydrochloride	0.4528
sodium citrate	0.4402
L-aspartic acid	0.4192
L-proline	0.3878
potassium citrate	0.3668
sodium phosphate dibasic	0.2851
L-tryptophan	0.2587
L-serine	0.2159
choline bitartrate	0.2154
L-alanine	0.1937
glycine	0.1886
potassium sorbate	0.1467
polyglycerol esters of fatty acids	0.1308
taurine	0.08300
vitamin E acetate	0.05659
ascorbic acid	0.05072
L-carnitine	0.04150
biotin	0.01761
zinc sulfate	0.01572
ferrous sulfate	0.01404
niacinamide	0.01199
vitamin A palmitate	0.01006
calcium pantothenate	0.006749
cyanocobalamin	0.003668
copper gluconate	0.003668
manganese sulfate	0.002369
folic acid	0.002348
vitamin K	0.002306
vitamin D	0.001761
pyridoxine hydrochloride	0.001467

potassium iodide 0.001149
riboflavin 0.001048
thiamin hydrochloride 0.000950
chromic acetate 0.000179
sodium molybdate 0.000159
sodium selenite 0.000055.

INCL INCLM: 514/021.000
INCLS: 514/002.000; 424/439.000; 424/600.000; 424/679.000; 424/709.000; 426/064.000; 426/072.000; 426/073.000
NCL NCLM: 514/021.000
NCLS: 424/439.000; 424/600.000; 424/679.000; 424/709.000; 426/064.000; 426/072.000; 426/073.000; 514/002.000
IC [6]
ICM: A61K038-01
ICS: A61K033-14; A23L001-202; A23L001-30
EXF 514/2; 514/21; 514/773; 514/777; 424/439; 424/600; 424/709; 424/679; 426/64; 426/72; 426/73; 426/800; 426/801; 426/810
ARTU 185
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 23 OF 33 USPATFULL
AN 95:64953 USPATFULL
TI Phospholipids containing **omega-3-fatty acids**
IN Larsson-Backstrom, Carin, Stockholm, Sweden
PA Pharmacia AB, Sweden (non-U.S. corporation)
PI US 5434183 19950718 <--
WO 9221335 19921210
AI US 1993-157024 19931229 (8)
WO 1992-SE333 19920519
19931229 PCT 371 date
19931229 PCT 102(e) date
PRAI SE 1991-1642 19910530
DT Utility
FS Granted
REP US 4820731 Apr 1989 514/549.000 Mascioli et al.
AT 333437 Feb 1974
EP 304603 Mar 1989
DE 3347269 Jul 1985
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SE 8705122 Jun 1989
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EXNAM Primary Examiner: Raymond, Richard L.
LREP Pollock, Vande Sande & Priddy
CLMN Number of Claims: 24
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 3 Drawing Page(s)

AB The invention relates to an emulsion which comprises phospholipids containing **omega-3-fatty acids** such as DHA and EPA in a high amount and a vegetable oil and/or marine oil. It also relates to the use of phospholipids containing the **omega-3-fatty acids** such as DHA and EPA in high amount for the manufacturing of a nutritive emulsion giving low serum triglyceride and cholesterol levels and for the manufacturing of a medicament with anti-**inflammatory** and immunosuppressive effects. The invention also discloses phospholipids containing **omega-3-fatty acids** such as DHA and EPA with therapeutic effects such as effects on **inflammatory** and immunologically active cells, e.g. rheumatoid arthritis and sepsis and an effect on normal brain and retina development and function and cardiovascular diseases. Also disclosed are pharmaceutical and nutritive compositions as well as lipid particles comprising the phospholipids.

SUMM This application is a 371 of PCT/SE92/00333, filed May 19, 1992.

Introduction

The present invention relates to emulsions containing phospholipids of marine and/or synthetic origin with a high mount of (at least 30% (w/w) of its total fatty acid content) omega-3 (hereinafter written as) w3-fatty acids, with docosahexaenoic acid, 22:6w3 (hereinafter written as DHA) and eicosapentaenoic acid, 20:5w3 (hereinafter written as EPA) in combination, as well as the use of these phospholipids. The phospholipids are used as emulsifiers, nutritive substrates, as a pharmacologically active agent in an emulsion, as a component in pharmaceutical compositions or as a component in lipid particles.

Background of the Invention

It is known that lipid emulsions can be used intravenously to constitute an energy dense caloric source and a source of essential fatty acids for those patients who have difficulties in using orally administered nutrition.

Since the beginning of the 1960s, fat emulsions which are intended for intravenous nutrient supply and which exhibit insignificant secondary effects have been available (Wretlind, A. Development of fat emulsions, JPEN 5: No 3,230-35, 1981).

This developmental work has investigated the effect of emulsions which contain a number of different fats, such as soy bean oil, maize oil, safflower oil, cottonseed oil etc. and different emulsifiers, such as soy bean phospholipids, egg yolk phospholipids etc.

Emulsions for nutritive or therapeutic use are, for example, described in U.S. Pat. No. 4,168,308.

The nutritive emulsions now most commonly used contain a vegetable oil such as soybean oil and/or safflower oil, an emulsifying agent such as an egg yolk phospholipid and water together with glycerol. An example of

such an emulsion is Intralipid.RTM., manufactured since 1962 and sold by Kabi Pharmacia AB. Intralipid.RTM. 10% contains 10% oil as soy bean oil and 1,2% egg yolk phospholipids.

Different fatty acids in the lipids have different physiological, biochemical and pharmacological properties and during the last years great interest has been concentrated on the importance of the w3-fatty acids, containing 18-22 carbon atoms.

The w3-fatty acids eicosapentaenoic acid (20:5w3, EPA) and docosahexaenoic acid (22:6w3, DHA) are essential fatty acids in man. Besides their nutritional value, they are also known to possess pharmacological effects. The most known and important are the cardiovascular effects, the beneficial effects on **inflammatory** and autoimmune diseases and the necessity of these fatty acids for the normal development of brain and retina functions.

These effects have such unimportance that a lot of work has been done to find good nutritional compositions containing a high mount of w3-fatty acids. See e.g. WO 87/02247 (Baxter) and U.S. Pat. No. 4,820,731 (New England Deaconess Hospital) in which marine oils are used which contain a high amount of the w3-fatty acids EPA and DHA.

The patient also needs **omega-6 fatty acids** (hereafter written as w6-fatty acids) which are found, for example, in vegetable oils. Nutrients given to patients should therefore also contain an appropriate vegetable oil. Infusion of lipid emulsions containing w6-fatty acids results, however, in a raised level of cholesterol and triglycerides in some patients, which should be avoided.

Until now some patients depending on parenteral nutrition have not been able to avoid a certain increase of total cholesterol and triglycerides when an emulsion containing mainly w6-fatty acids is given. w6-Fatty acids also increase the level of eicosanoides and leucotrienes, which when overproduced in some patients, e.g. with overactive **inflammatory** and immunological reactions, may have deleterious effects.

Phospholipids containing EPA or DHA are known as being useful in various fields, such as foods, cosmetics, medicines, agriculture etc. and different methods for their-manufacture have been disclosed. See e.g. JP 2097393 and JP 1050890.

The use of phospholipids, containing EPA or DHA, as emulsifier for an EPA-triglyceride-emulsion and a DHA-triglyceride-emulsion, respectively, has been investigated by Hamazaki T et al in Biochem and Biophys Res Comm. Vol 151, No 3, 1386-1394, 1988; in LIPIDS Vol 22 No 12, 1031-1034, 1987 and in Thrombosis Research Vol 44, 1986, (673-682). Hamazaki found, for example, that both the EPA and DHA levels in platelets and RBC (red blood cell) membranes increased significantly when either the EPA or the DHA emulsion, respectively, were infused intravenously for a short time. Blood lipids remained unchanged, except for free fatty acids which decreased. Platelet aggregation and leucocyte adhesion were depressed mainly afar administration of the EPA containing emulsion.

The Swedish patent application SE 8705122-3 is related to a method for

manufacturing fatty emulsions with phospholipids from eggs as an emulsifier consisting of at least 10% (w/w) w3-fatty acids, wherein the phospholipids are derived from eggs of animals fed with a diet rich in marine oils.

This method of incorporating w3-fatty acids in phospholipids is however provided with natural limitations. It has not been possible to exceed about 15% (w/w) of w3-fatty acids in phospholipids derived by this method, with the level of EPA about 2% (w/w), which in the conventional lipid emulsions described is to be considered to be below the level for therapeutic effects.

The egg phospholipids described in the above mentioned Swedish patent application does not necessarily have the therapeutic effects presumed.

Since there is evidence which suggests that dietary EPA can provide some clinical benefit in treatment of **inflammatory** diseases (Salmon, n-3 News, Vol II (3) 1987), it is important to have a high mount of DHA as well as EPA in the phospholipids.

DE 3347269 describes a method of how to synthetically manufacture lecithine and lyso-lecithine containing EPA and/or DHA, but does not reveal anything about the total amount incorporated of w3-fatty acids or the therpeutical use of the product.

No one has, however, investigated the biological effects and potency of phospholipids, and compared them with those of triglycerides, containing the w3-fatty acids such as EPA and DHA in combination in a high amount in an emulsion with an w6-fatty acid containing vegetable oil or the therapeutic use of phospholipids containing the w3-fatty acids such as EPA and DHA in combination in high amount

DESCRIPTION OF THE INVENTION

We have now found that when using the w3-fatty acids DHA and EPA in high amount in combination in phospholipids from marine or synthetic origin, instead of using them as triglycerides, but together with a vegetable oil containing w6-fatty acids in a nutritive lipid emulsion, the amount of serum cholesterol and triglycerides are surprisingly lower than with the same amount of w3-fatty acids given as fish oil. By using this origin of w3-fatty acids in phospholipids the amount of all the important w3-fatty acids, together with all the important and essential w6-fatty acids was increased in biological membranes. Furthermore, the incorporation of w3-fatty acids into biological membranes is unexpectedly increased.

It is also totally unexpected that the incorporation biological membranes of w3-fatty acids as well as w6-fatty acids is more efficient, and the potency is higher, by using the w3-fatty acid-rich phospholipid (according to the invention) than with the same amount of w3- and w6-fatty acids given as marine oil in emulsion or as vegetable oils in emulsion.

Most surprisingly and important is the finding that DHA is specifically increased in membrane phospholipids. This is of utmost importance since DHA, being the most important w3-fatty acid in phospholipids in biological membranes, does not compete with and decrease the level of arachidonic acid, the most important w6-fatty acid in phospholipids in biological membranes, as much as EPA does. In cholesterol esters also the level of EPA and alpha-**linolenic acid** are increased.

In all lipid fractions the level of arachidonic acid, which is the most important w6-fatty acid in biological membranes, is maintained constant.

This is in contrary to the reduction of arachidonic acid in biological membranes, which is observed when using therapeutic doses of marine oils, containing a high amount of EPA. After the administration of fish phospholipids (according to the invention), however, even the levels in triglycerides of the w6-fatty acids are increased.

This indicates that the metabolism of arachidonic acid to eicosanoides is reduced and thus the w6-fatty acids are in good balance with the w3-fatty acids and are spared as important components in biological membranes.

The findings are of utmost interest for nutritive emulsions as the amount of total serum cholesterol and triglycerides should be kept as low as possible and the levels of w6- and w3-fatty acids in biological membranes should be kept in balance.

The findings are also of utmost interest to obtain normal and well balanced levels of essential fatty acids, w3-fatty acids as well as w6-fatty acids, for nutritive emulsions to premature/newborn babies and in long-term TPN (total parenteral nutrition). The specific increase in DHA in phospholipids together with the increase of all important w3- and w6-fatty acids in cholesterol esters and triglycerides fulfill the nutritive requirements of a well-balanced, increased level of W3-fatty acids as well as w6-fatty acids. Such emulsions can thus be useful nutritionally for example, in long term total parenteral nutrition (TPN) and in prematures/newborn patients, who need w3-fatty acids as well as w6-fatty acids for normal brain and retinal development.

Love et al, Annals of the Rheumatic Diseases, 1990, 49, pp 611-614 has shown that egg phospholipids are accumulated in immunologically active cells. Billiar T R et al ("Fatty add intake and Kupffer cell function; Fish oil alters eicosanoid and monokine production to endotoxin stimulation" Surgery, 104, 343-349 1988) has shown that w3-fatty acids are incorporated into and have anti-**inflammatory** effects on Kupffer cells, when fish oil with w3-fatty acids was given orally. We have now surprisingly found that w3-fatty acid containing marine phospholipids accumulate in Kupffer cells and that DHA from the marine phospholipids is incorporated in membrane phospholipids with an unexpectedly high specificity and potency. Also the other essential w3-fatty acids, EPA and alpha-**linolenic acid**, which are increased in cholesterol esters, as well as the essential w6-fatty acids are increased in neutral lipida. The increase in membrane lipids of w3-fatty acids as well as of w6-fatty acids show that the potency of the w3-fatty-adds is significantly higher after administration of w3-fatty acid containing marine phospholipids together with vegetable oil in emulsion (the invention) than after administration of comparable amounts of w3- and w6-fatty acids in fish oil and vegetable oil, respectively, in an emulsion.

The phospholipids according to the invention accumulate in Kupffer cells and can thus be used to reduce the w6-/w3-fatty acid ratio in stimulated immunologically active cells for the treatment of diseases with increased **inflammatory** and immunological reactions, e.g. sepsis, rhetmatoid arthritis or other autoimmune and **inflammatory** diseases.

The invention thus relates to an emulsion comprising vegetable oil and/or fish oil which contains phospholipid with the **omega**-

3-fatty acids DHA and EPA in high amount in combination which can be used as a nutritive emulsion to meet the requirement of essential fatty acids, e. g. in long-term TPN and for premature/newborn babies. This emulsion can also be used for therapeutic purposes for a better w3-/w6-fatty acid balance, with serum lipid lowering and anti-**inflammatory** effects, effects on hemostatis, and in higher dosages immunosuppressive effects.

It also relates to the use of phospholipids containing w-3-fatty acids for the manufacturing of a medicament,with anti-**inflammatory** and immunosuppressive effects and the use of phospholipids with the **omega 3-fatty acids** DHA and EPA in combination for the manufacture of a nutrition emulsion giving low serum triglyceride and cholesterol levels and a more balanced w6-/w3-fatty acid ratio and with anti-**inflammatory** and immunosuppressive effects and effects on hemostatis:

The invention also relates to phospholipids containing the **omega -3-fatty acids** DHA and EPA with therapeutic effects on diseases with overproduction of eicosanoids in **inflammatory** and immunologically active cells, on rheumatoid arthritis, **inflammatory** situations and on the development and function of normal brain and retina. Another aspect of the invention is to use phospholipids with EPA and DHA in a high mount in combination with drugs with similar effects or used for diagnostic purposes.

The emulsion could comprise 0,5-40% (w/v of total emulsion) oil, preferably 5-30% (w/v), such as soybean oil, coconut oil, cottonseed oil, safflower oil, sunflower seed oil, linseed oil, borage oil, blackcurrent seed oil, canola oil, marine oil or a mixture of these. The mount of the phospholipids according to the invention could be 0,1-30% (w/v of total emulsion), preferably 0,1-10% (w/v). The phospholipids containing w3-fatty acids could be of marine or synthetic origin.

Other phospholipids such as egg yolk or soybean phospholipids and/or synthetic emulsifiers can also be included as complements in the emulsion. The total amount of emulsifier is preferably 0,1-30% (w/v of total emulsion).

The emulsion can also contain other components which normally are incorporated in emulsions e.g.: monoglycerides of fatty acids, components for adjusting of isotonic properties such as glycerol, anti-oxidants such as alpha-tocopherol, components for adjusting stability such as amino acids and carbohydrates such as fructose and glucose etc. It can also contain one or more bioactive compounds to be administered.

The preparation of the emulsion is carried out in a conventional manner. Thus the lipids are mixed with the aqueous phase, phospholipids according to the invention and optionally other emulsifiers and auxiliary agents in a suitable mixing device. The blend is thereafter homogenized to a desired particle size. The ways to adjust the emulsion to a suitible particle size is well known to a person skilled in the art.

Our findings are of utmost interest for nutritive emulsions to keep the amount of total cholesterol and triglycerides as low as possible for the patient and the balance of the ratio w6/w3-fatty acids in biological membranes optimal e. g. for newborn/premature infants, in long-term TPN and in situations with stimulated **inflammatory** and immunological reactions.

The phospholipids according to the invention are also concievable as components in lipid particles such as liposomes or any other mono-, bi- or multilayered vesicle.

The means and methods of how to use phospholipids to prepare such vesicles are well-known to anyone skilled in the art since numerous papers and patents have been published in this technical field (an overview of liposome preparation can be found in Drug Dev Ind Pharm 15 (19), 1523-54, 1989).

An aspect of the invention is to use the phospholipids with a high content of w3-fatty acids in the preparation of various lipid vesicles, either to deliver one or more bioactive components, or to be an administration form in itself for the highly therapeutically potent phospholipids.

Additional bioactive components can be enclosed in the vesicles or be parts of their membranes or can in certain cases be conjugated to the membrane molecules.

These systems can be taylored individually for each bioactive molecule and depend on the net charge, molecular weight and the number of hydrophilic or hydrophobic groups on the molecules.

The bioactive compounds may be such that potentiates the therapeutical effects of the administered w3-fatty acids or any other drug, which is appropriate to deliver.

A bioactive Compound used in combination with the vesicles can also be a ligand with affinity to a biological receptor to create a more specific drug targeting system.

The vesicles may also be used for diagnostic purposes and the bioactive compound can in such cases be a labeled or signal-carrying molecule.

The vesicles prepared from the phospholipids according to the invention can be administered in conventional manners in pharmaceutical or diagnostical preparations. The additional ingredients for adapting the preparations for oral, buccal, parenteral, intraocular, nasal, pulmonary, rectal, or transdermal use are well-known for anyone skilled in the art.

The phospholipids according to the invention can also be administered in any oral, parenteral, intraocular, nasal, pulmonary, rectal or transdermal preparation in combination with conventional carriers and/or diluents. The administration forms can also, when appropriate comprise other adjuvants and enhancers for increasing or controlling membrane penetration such as monoglycerides and compounds with surface active properties.

DRWD FOLLOWING FIGURES ARE INCLUDED

FIG. 1 which shows the level of serum cholesterol (S-CHOL) after administration of different emulsions and solutions. See example 4.

FIG. 2 which shows the level of serum triglycerides (S-TG) after administration of different emulsions and solutions. See example 4.

FIGS. 3-5 show the level in liver phospholipids (PL;FIG. 3), in liver cholesterol esters (CE; FIG. 4) and in liver triglycerides (TG; FIG. 5) of the most important w3-fatty acids DHA (22:6w3), EPA (20:5 w3), alphalinolenic acid (18:3 w3) and the w6-fatty acids dihomo-gamma-

linolenic acid (20:3 w6) and gamma-**linolenic acid** (18:3 w6) after administration of different emulsions and solutions. See example 4. Statistically significant increase ($p<0.05$, ANOVA) after administration of fish-phospholipid containing emulsion (*, F-PLem) compared to fish oil emulsion (FOem) and Intralipid (IL) and after FOern (+) compared to the other groups.

FIG. 6 shows the level of linoleic acid (18:2 w6) and

FIG. 7 shows the level of arachidonic acid (20:4 w6) in liver lipids after administration of different emulsions and solutions. See example 4. For explanation of abbreviations, see FIGS. 3-5.

DETD Various modifications and equivalents of the emulsion will be apparent to one skilled in the art without departing from the spirit or scope of the invention. It is therefore to be understood that the invention is not to be limited to the specific examples and embodiments disclosed herein.

Fish phospholipids can be manufactured in different ways and in the following only one example of such a manufacturing is given.

EXAMPLES

Example 1

Preparation of fish phospholipids

1,5 kg fishmeal was extracted two consecutive times with 6 l and 3 l 95% ethanol. After filtration and pooling, ethanol was evaporated from the extract (vacuum, 40.degree. C.). 134 g was left (22% of this was insoluble matter). This residue was dissolved in 1 volume petroleum ether, filtered, precipitated in four volumes -20.degree. C. acetone, filtered and dissolved in petroleum ether. This last precipation and dissolution was made twice. After precipitation again in four volumes of acetone, the solution was filtered and freeze-dried in a nitrogen atmosphere. The yield was 27 g.

The prepared fish phospholipid had the following fatty acid content in %:

14:0	Myristic acid	2
16:0	Palmitic acid	26
16:1	Palmitoleic acid	2,1
18:0	Stearic acid	3,0
18:1	Oleic acid	11,3
18:2	Linoleic acid	1,1
20:1	Eicosenoic acid	1,6
20:4	Arachidonic acid	1,2
20:5	EPA	10,6
22:6	DHA	32,4

Total amount of fatty acids: 100% (w/w)

Example 2

Preparation of a fish phospholipid emulsion according to the invention.

The fish phospholipid from example 1 was used for the manufacturing of an emulsion containing:

100	g	soybean oil
12,0	g	fish phospholipid
22,2	g	glycerol
860	g	Aq. ad inject.
3,0	ml	NaOH 1M

The ingredients were mixed in a "Turrax-mixer" and thereafter homogenized in a "Moulin-Gaulin Homogenizer".

The soybean oil used had the following fatty acid content in %:

16:0	Palmitic acid	11
18:0	Stearic acid	4
18:1	Oleic acid	23
18:2	Linoleic acid	55
18:3	Alfa-**linolenic acid**	7

Total amount of fatty acids: 100% (w/w)

The total amount of w3-fatty acids is 12,2 g/l emulsion and the ratio w6-/w3-fatty acids is 4,5:1.

Example 3

Preparation of an emulsion contains fish oil and egg yolk phospholipids.

This emulsion was prepared according to the method described in Example 2, and contains a similar amount of w3-fatty acids and a similar w6/w3-fatty acid ratio as in Example 2.

The emulsion contained:

Fish oil	10,o	g
Soy bean oil	90,0	g
Egg yolk phospholipid	12,0	g
Glycerol	22,2	g
Aq. ad inject.	860	g
NaOH, 1M	3,0	ml

As antioxidant vitamin E (alpha-tocopherol) was added to the emulsion
The fish oil used had the following fatty acid content in %:

14:0	Myristic acid	6,3
16:0	Palmitic aid	14,7
16:1	Palmitoleic acid	7,3
18:0	Stearic acid	2,6
18:1	Oleic acid	8,9
18:1	Vaccenic acid	3,1
18:2	Linoleic acid	1,1
18:3	**Linolenic acid**	0,7
18:4	Stearidonic acid	2,6
20:1	Eicosenoic acid	1,5
20:4	Arachidonic acid	1,4
20:5	EPA	17,8
22:1	Docosaenoic acid	2,2
22:5	Docosapentaenoic acid	

		2,9
22:6	DHA	13,5

Total amount of fatty acids: 100% (w/w).

The egg yolk phospholipids used had the following fatty acid content in %:

14:0	Myristic acid	0,2
16:0	Palmitic acid	31,5
16:1	Palmitoleic acid	1,2
18:0	Stearic acid	14,1
18:1	Oleic acid	28,0
18:2	Linoleic acid	12,4
20:1	Eicosenoic acid	0,2
20:4	Arachidonic acid	4,2
22:6	DHA	5,8

The total mount of w3-fatty acids is 10,8 g/l emulsion and the ratio of w6-/w3-fatty acids is 4,8:1.

Example 4

Comparative example, (Fish phopholipid-fish oil)

The purpose of this example was to investigate the effects of the fish phospholipid preparation in an emulsion according to the invention and to compare it with different emulsions such as a fish oil emulsion containing the same amount of w3-fatty acids, Intralipid.RTM. and also to compare it with fish phospholipids in water solution and physiological saline solution.

Male sprague Dawley rats, with weight on arrival of 170-190 g were used.

The rats were placed individually in cages and provided with a preweighed small leather harness. The i.v. catheter was inserted 7-8 days later under anaesthesia. After the operation the animals were placed in the infusion room. Another 4 days were allowed for recovery.

After surgery the rats were provided with grounded laboratory stock diet R3 (Ewos AB; Sodertalje, Sweden) and tap water ad libitum. During the entire test period the rats were provided with grounded laboratory stock diet R3 and tap water ad libitum.

The rats were randomized using a random unit into experimental groups A-E.

Six rats were used in each experimental group

All groups received 50 ml/kg body weight (b.w.)/day. Infusions were administrated intravenously via a permanent central vein catheter during 20 h/day, normally from 1 p.m. to 9 a.m., via IMED volumetric pumps.

Food consumption was recorded on a 24 h basis. The general appearance of the rats was recorded. The infusions were given during 9 consecutive. days. On day 10 the infusions were stopped at 7.00 a.m. and the oral food withdrawn.

The groups A-E were given the following infusions:

A: 10% Fat emulsion according to Example 2 (F-PLem, the Invention)

B: -"- according to Example 3 (FOem)

C: 10% Intralipid .RTM., containing 10% (w/v) oil as soy bean oil and 1,2% egg yolk phospholipid

D: 1.2% Phospholipid Solution containing:

Fish phospholipids	12,0	g
Glycerol	22,2	g
Aq. ad inject.	967	g
NaOH 1M	3	ml

E: 0.9% NaCl-solution

The amount of w3- and w6-fatty acids were as follows:

		g/l tot w3-FA	tot w6-FA	w6/w3
A =	Ex 2 (Invention)	12,2	55,2	4,5
B =	Ex 3 (Fish oil)	10,8	51,6	4,8
C =	Intralipid	7,7	56,8	7,4
D =	Fish phospholipid solution	4,8	0,3	0,1

Approx. 2 hours after stopping the infusions, the rats were anaesthetized with Mebumal.RTM. (60 mg/kg). The blood samples were collected for analysis of serum lipids.

Serum lipids: From 1 ml blood, serum was taken for analysis of serum triglycerides and serum cholesterol and frozen at -70.degree. C. until analysis. Serum cholesterol was measured enzymatically. Serum triglycerides were measured enzymatically after eliminating free glycerol and enzymatic hydrolysis.

Histopathology; liver, kidneys, heart, lungs, spleen and thymus were excised, weighed and prepared for histopathological examination by embedding in paraffin and sectioning at 4-5 micrometer, then staining with haematoxylin-eosin. Frozen sections from all the tissues stained with Oil Red O for fat were also examined.

Fatty acid profile in liver lipids. The remaining liver tissue from rats in Groups A-C was used for measurement of fatty acid profile in neutral lipids and phospholipids(PL). The neutral lipid fractions examined were cholesterol esters (CE) and triglycerides (TG). The lipid material was extracted and the fatty acids derivatized and analyzed (GLC) using conventional methods.

The resulting data on serum lipids are shown in FIG. 1 (level of serum cholesterol, S-CHOL) and FIG. 2 (level of serum triglycerides, S-TG). The levels of the most important fatty acids in liver lipids are shown in FIGS. 3-7. The results are expressed as mean values .+-.SEM for the different emulsions and solutions.

Results and conclusions

FIG. 1 clearly shows that the serum cholesterol level is very low when the emulsion according to the invention is used (*A<B, C; $p<0.05$, ANOVA). The most surprising effect, however, is the result when comparing the serum triglyceride levels. See FIG. 2. That level is surprisingly low (0,30 mmol/l) and only half of the value when Intralipid.RTM., fish phospholipids in a solution or NaCl solution (0,60 mmol/l) were used (*A<B, C, D, E; $p<0.05$, ANOVA).

It is absolutely unexpected and unknown, that the small amount of w3-fatty adds which can be derived from phospholipids in a lipid emulsion, can exert physiological effects. The same amount of w3-fatty acids in triglycerides (Group B) does not exert the same biological effects or effects on fatty acid incorporation. Since the phospholipid/triglyceride ratio is about 1:10 in a 10% lipid emulsion and 1:20 in a 20% lipid emulsion, and the threshold level for biological effects for fish oil, containing about 30% w3-fatty acids, is at least 10% of the oil phase, the amount of w3-fatty acids in the phospholipid part of a lipid emulsion should be at least 30% (w/w of total fatty acids) to be expected to exert biological effects.

Thus giving an emulsion, according to the invention allows the patient to receive all essential fatty acids such as w6- and w3-fatty acids and still remain at a very low level of serum cholesterol and serum triglycerides.

The histopathological data showed that the emulsions with fish oil or fish phospholipids were accumulated mainly in the Kupffer cells in the liver, whereas Intralipid.RTM. was accumulated as well in hepatocytes. Fish phospholipids in water solution were however accumulated only in Kupffer cells in the liver and in similar cells in the spleen. These cells are immunologically active. This specific accumulation to immunologically active cells facilitates an effect of w3-fatty acids on **inflammatory** and immunological reactions. This is of importance when using fish phospholipids for **inflammatory** or immunological diseases such as rheumateid arthritis and sepsis (Love et al., 1990 and Billiar et al.,1988) or to reduce the incidence of arterosderosis.

The fatty acid pattern in liver phospholipids and liver cholesterol esters show that EPA and DHA are incorporated better after treatment with the emulsion according to the invention when compared to the fish oil emulsion containing the same amount of w3-fatty acids.

The changes induced by fish phospholipid containing emulsion (F-PLem), fish oil containing emulsion (FOem) or Intralipid (IL) in fatty acid pattern in liver phospholipids (PL), cholesterol esters (CE) and triglycerides (TG) are shown in FIGS. 3-7. In the phospholipid fraction (FIGS. 3 and 6), the only changes seen were increases in DHA, induced by F-PLem (invention, *) $p<0.05$, ANOVA) and in EPA, induced by FOem (+$p<0.05$, ANOVA).

The specific increase in DHA in phospholipids, the main pool for DHA, is of importance for brain and retina development.

The uptake of and the level in biological membranes of polyunsaturated fatty acids, especially DHA in brain, is well correlated with that in liver (Anderson and Connor,Lipids 1988, 23(4), 286-290) and in heart (Swanson et al., British Journal of Nutrition, 1988, 59, 535-545). Thus a similar enhancement of uptake in brain and heart is expected following administration of the invented emulsion compared to the fish oil

emulsion. Therefore the invented emulsion can be used also for normal brain and retina development and for cardiovascular diseases.

In the liver cholesterol ester fraction (FIGS. 4 and 6) F-PLem increased DHA, EPA and linoleic acid (18:2w6), compared to the FOem- and the IL-groups, and .alpha.-**linolenic acid** (18:3w3), compared to the IL-group. Thus, w3-fatty acids administered in the phospholipid form, are more effectively incorporated in membrane lipids (phospholipids and cholesterol esters) than w3-fatty acids in the glyceride form (fish oil) are. The main functions of the essential fatty acids are thus also the w3-fatty acids are exerted in the membrane lipids.

No decrease in any of the w6-fatty acids could be seen in the F-PLem group. On the contrary, the w6-fatty acids linoleic acid (18:2 w6, FIG. 6), gamma-**linolenic acid** (18:3w6) and dihomo-gamma-**linolenic acid** (20:3w6), as well as alpha-**linolenic acid** (18:3w3) (FIG. 5) were increased in the triglyceride fraction, compared to the FOem- and IL-groups. The level of arachidonic acid (20:4w6) remained unchanged in all lipid fractions (FIG. 7). This finding is of significance because of the importance of arachidonic acid in biological membranes.

Since the w3-fatty acid containing phospholipids are taken up more in Kupffer cells than in hepatocytes, as shown in the invention, the relative increase in incorporated w3-fatty acids is expected to be even higher in immunologically active cells (Kupffer cells and other macrophages) than in the whole liver. This is of importance for the anti-**inflammatory** and immunosuppressive effects exerted by the w3-fatty acids. The biological effects obtained by the w3-fatty acids in phospholipids can be used for therapeutic purposes as such or in combination with drugs with similar effects and included in the phospholipid vesicles (liposomes).

Conclusions

We have shown that marine phospholipids in an emulsion according to the invention result in surprisingly lower serum triglyceride and serum cholesterol levels when compared to fish oil emulsion, containing a similar amount of w3-fatty acids and a similar w6/w3-fatty acids ratio. Marine phospholipids induce more effective incorporation of w3-fatty acids in biological membranes than fish oil, containing a comparable amount of w3-fatty acids, in an emulsion.

These results show that a very favorable fatty acid pattern in membrane lipids is obtained, with an increase in all important w3-fatty acids as well as in the w6-fatty acids linoleic, gamma-linolenic and dihomo-gamma-**linolenic acids**. The invented w3-fatty acid containing phospholipid is therefore important in all situations with increased need of all essential fatty acids, since it makes it possible to increase the w3-fatty acids (from the invented phospholipid) as well as the w6-fatty acids (in vegetable oils) in a well-balanced pattern.

These results have implications for the use of w3-fatty acid containing phospholipids in vegetable oil emulsions for a more effective utilization and incorporation of w3- and w6-fatty acids. The use of w3-fatty acid containing phospholipids also allows a more specific incorporation of w3fatty acids in immunologically active cells.

The invention may have implications specifically for situations with

increased level of serum lipids, increased **inflammatory** response and increased immunological activity and also for the normal development of the brain and retina.

The biological effects obtained by the w3-fatty acids in phospholipids can be used for therapeutic purposes as such or in combination with drugs with similar effects and included in the phospholipid vesicles (liposomes).

CLM What is claimed is:

1. An emulsion comprising vegetable oil and/or marine oil, an aqueous phase and phospholipids as emulsifier characterized in that the phospholipids are of marine and/or synthetic origin and contain **omega-3-fatty acids** in an amount of at least 30% (w/w).

2. The emulsion of claim 1 which comprises a vegetable oil.

3. An emulsion according to claim 1 characterized in that the **omega-3-fatty acids**, present in an amount of at least 30% (w/w), are DHA and EPA.

4. An emulsion according to claim 3 characterized in that the amount of oil is 0,5-40% (w/v) and the amount of phospholipids containing the **omega-3-fatty acids** is 0,1-20% (w/v).

5. An emulsion according to claim 1 characterized in that the amount of oil is 0.5-40% (w/v) and the amount of phospholipids containing the **omega-3-fatty acids** is 0.1-30% (w/v).

6. An emulsion according to claim 5 characterized in that it contains one or more bioactive compounds.

7. Phospholipids of marine and/or synthetic origin containing **omega-3-fatty acids** in an amount of at least 30% (w/w) with therapeutic effect.

8. Phospholipids according to claim 7 characterized in that **omega-3-fatty acids** are DHA and EPA in an amount of at least 30% (w/w).

9. A mono-, bi and/or multilayered vesicle or any mixture thereof characterized by its content of phospholipids containing the **omega 3-fatty acids** in an mount of more than 30% (w/w).

10. A mono-, bi and/or multilayered vesicle or any mixture thereof according to claim 9 characterized in that the **omega 3 -fatty acids** are EPA and DHA.

11. A composition comprising the vesicles according to claim 9, campers and/or diluents and optionally one or more bioactive compound(s) combined with the vesicles.

12. A composition comprising the vesicles according to claim 10, carriers and/or diluents and optionally one or more bioactive compound(s) combined with the vesicles.

13. A pharmaceutical composition comprising the phospholipids according to claim 7 and carriers and/or diluents for adapting it to oral, nasal, pulmonary, rectal, ocular, transdermal or parenteral administration optionally in combination with one or more bioactive compound(s).

14. The pharmaceutical composition of claim 13 wherein the phospholipid

is in an amount sufficient for anti-**inflammatory** and/or immunosuppressive effects.

15. A pharmaceutical composition comprising the phospholipids according to claim 8 and carriers and/or diluents for adapting it to oral, nasal, pulmonary, rectal, ocular, transdermal or parenteral administration optionally in combination with one or more bioactive compound(s).

16. A nutritive composition comprising the phospholipids according to claim 7 and carriers and/or diluents.

17. The nutritive composition of claim 16 being capable of giving low blood triglyceride and cholesterol levels.

18. A nutritive composition comprising the phospolipids according to claim 8 and carriers and/or diluents.

19. A method for treating a patient in need of anti-**inflammatory** and/or immunosuppressive effects which comprises administering to said patient an effective amount of the phospholipids of claim 7.

20. A method for treating a patient in need of anti-**inflammatory** and/or immunosuppressive effects which comprises administering to said patient an effective amount of the phospholipids of claim 8.

21. A method for treating a patient suffering from rheumatoid arthritis or sepsis which comprises administering to said patient an effective amount of the phospholipids of claim 7.

22. A method for treating a patient suffering from rheumatoid arthritis or sepsis which comprises administering to said patient an effective amount of the phospholipids of claim 8.

23. A method for promoting normal brain and/or retina development and function in a patient which comprises administering to said patient an effective amount of the phospholipids of claim 7.

24. A method for promoting normal brain and/or retina development and function in a patient which comprises administering to said patient an effective amount of the phospholipids of claim 8.

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ARTU 129
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 24 OF 33 USPATFULL
AN 95:38699 USPATFULL
TI Compositions and methods for inhibiting **inflammation** and adhesion formation
IN Bockow, Barry I., 16122-8th Ave. S.W., Ste. D3, Seattle, WA, United States 98166
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EXNAM Primary Examiner: Henley, III, Raymond
LREP Seed and Berry
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ECL Exemplary Claim: 1
DRWN No Drawings
AB There is disclosed compositions and methods for inhibiting **inflammation** and/or adhesion formation in a patient. The compositions comprise omega-3 and/or **omega-6 fatty acids**, a nonionic surfactant, and a pharmaceutically acceptable carriers or diluents. The omega fatty acid compositions may optionally contain cyclooxygenase inhibitors and other additives and preservatives such as dextrose and vitamin A. The methods of inhibiting **inflammation** and/or adhesion formation in a patient comprise administration of an effective quantity of a composition to a body cavity of the patient.

SUMM TECHNICAL FIELD

This invention relates generally to compositions and methods for inhibiting **inflammation** and/or adhesion formation in patients and, more specifically, to compositions containing omega fatty acids and methods for their administration.

BACKGROUND OF THE INVENTION

The prevention or inhibition of **inflammation** and adhesion formation is of significant concern to medical professionals.

Adhesion formation is a final common physiological result of **inflammation** from any cause. Although it is commonly seen following surgery, it can also occur in many other clinical settings. In the post-operative setting, serious scar and/or adhesion formation can occur, greatly increasing patient morbidity. If this serious complication could be avoided, great savings could be realized in health care costs by reducing the need for future hospitalizations and therapeutic interventions. Furthermore, if adhesion formation is prevented, many chronic diseases could be prevented or at least mitigated.

In response to any tissue injury, the body will attempt to heal itself. The manner in which the body responds to an initial injury can frequently determine whether a person will return to normal health or

develop significant chronic disease. For example, adhesions are an undesirable biological response to tissue injury. They sometimes occur post-operatively, but may also occur after other forms of injury such as infection and trauma.

The healing process is mediated by the immune response. The immune system is a protective network that enables the body to ward off disease. For example, a microorganism, such as a bacteria or virus, can invade the body and thereby activate the immune system. Under normal circumstances, the host is protected when the invading microorganism is eliminated.

The body's host mechanism, in attempting to heal areas of injury, sometimes mounts an overly aggressive immune response. This undesirable reaction can lead to scar formation and/or adhesions. This "hyper" immune response is also the cause of a group of illnesses known as "autoimmune" diseases which include rheumatoid arthritis, systemic lupus erythematosis, thyroiditis, **inflammatory** bowel disease, allergies, and many chronic dermatologic diseases such as psoriasis and eczema. Recently researchers have presented evidence that atherosclerosis or "hardening of the arteries" is also mediated via this "hyper" immune response.

Often the immune response is associated with increased blood flow and increased vascular permeability. This causes the release of white blood cells, macrophages, platelets and other cellular elements to the surrounding tissues. These cells are the harbingers of **inflammation**. By blocking or restricting the immune response and by inhibiting certain cellular functions, the formation of excessive scar tissue can be inhibited. While adhesion formation results to some extent for all large **inflammations** or in instances where marked cell damage has occurred, adhesion formation generally results from an overly aggressive immune response mounted by the host in an attempt to heal the injured tissue.

Prostaglandins are a family of compounds which have been identified as playing a significant role in **inflammation**. Their biosynthesis is triggered by the release of arachidonic acid, a preliminary event in the immune response. Prostaglandins are produced throughout the body and are derived from enzymatic action on a common substrate, arachidonic acid. The first step in prostaglandin synthesis is the oxygenation of arachidonic acid by the enzyme cyclooxygenase. The oxygenated prostaglandin precursors are subject to further enzymatic processes which provide the various members of the prostaglandin family.

Closely related in structure and function to the prostaglandins are a family of compounds known as leukotrienes. Leukotrienes are also derived from arachidonic acid metabolism, but through the lipoxygenase pathway. Like prostaglandins, leukotrienes exhibit **inflammatory** properties.

Arachidonic acid is an essential fatty acid consisting of twenty carbon atoms and containing four carbon-carbon double bonds. By virtue of the position of the carbon-carbon double bond at the methyl (omega) end of the hydrocarbon chain, it is classified as an **omega-6 fatty acid**. A closely related family of fatty acids are the **omega-3 fatty acids**. In addition to double bond position, omega-6 and **omega-3 fatty acids** may also be distinguished by their origins. The precursors to these fatty acids are derived from botanical and/or marine plants which are in turn further metabolized in animals to

provide the long chain polyunsaturated acids. **Omega-6 fatty acids** may be found predominantly in land animals, while **omega-3 fatty acids** are abundant in fish.

In principle, any immune response may be modulated by stimulation or suppression--that is, immunomodulation may be accomplished through the use of immunostimulants or immunosuppressants (collectively referred to as immunomodulators). Therefore, immunomodulators may be effective in treating **inflammatory** conditions and preventing adhesion formation.

To this end, it is believed that inhibition of the enzymatic pathways which yield prostaglandins and leukotrienes would result in decreased production of these compounds and a consequent reduction in their **inflammatory** effects. The inhibitors of these enzymatic pathways are thus immunomodulators of the immune response.

As mentioned above, adhesion formation has been attributed to overly aggressive immune response. One class of compounds which has been identified as immunostimulants are interleukins. Interleukins are soluble immuno-enhancing glycoproteins produced by T-lymphocytes and have been commonly utilized as treatments to restore and/or bolster immune response in immunodeficient conditions. Accordingly, compounds which inhibit interleukin production are also immunomodulators. Such inhibitors are believed to suppress interleukin production and, consequently, immune response thereby effectively inhibiting both **inflammation** and adhesion formation.

Despite the great need to inhibit adhesion formation, current therapeutic options and preventive measures are of little or limited effectiveness. Accordingly, there is a need in the art for compositions which effectively modulate immune response and prevent, inhibit, or provide treatment for **inflammation** and adhesion formation. The present invention fulfills these needs, and provides further related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention is directed to compositions and methods for inhibiting **inflammation** and adhesion formation in warm-blooded animals, including humans (hereinafter referred to as patients) and, more specifically, to compositions containing omega fatty acids and methods for their administration.

In one aspect of this invention, compositions which contain omega fatty acids are disclosed. In one embodiment, a composition comprising an **omega-3 fatty acid** (a fatty acid derived from marine origins) is disclosed. The composition contains therapeutically effective amounts of the **omega-3 fatty acid** in combination with a nonionic surfactant and one or more acceptable carriers and/or diluents.

In another embodiment, a composition comprising an **omega-6 fatty acid** (a fatty acid derived from botanical origins) is disclosed. The composition contains therapeutically effective amounts of the **omega-6 fatty acid** in combination with a nonionic surfactant and one or more acceptable carriers and/or diluents.

In a further embodiment, a composition comprising both an omega-3 and an **omega-6 fatty acid** is disclosed. The composition contains therapeutically effective amounts of the omega-3 and **omega-6 fatty acids**

in combination with a nonionic surfactant and one or more acceptable carriers and/or diluents.

The omega fatty acid compositions of the present invention may further comprise cyclooxygenase inhibitors and other additives and preservatives such as dextrose and vitamin A.

In another aspect of the invention, methods for inhibiting **inflammation** and adhesion formation in a patient are disclosed. These methods comprise administration of an effective quantity of the compositions of the present invention to a body cavity of the patient.

Other aspects of the present invention will become evident upon reference to the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is generally directed to compositions and methods for inhibiting **inflammation** and adhesion formation in patients and, more specifically, to compositions containing omega fatty acids derived from marine and/or botanical sources, as well as methods for their administration. In addition to an omega fatty acid, the compositions of the present invention also contain a nonionic surfactant and a pharmaceutically acceptable carrier or diluent.

Although not intending to be limited to the following theory, it is believed that the compositions of the present invention effectively inhibit the syntheses of biochemicals which are ultimately responsible for **inflammation** and adhesion formation. In **inflammation,** these biochemicals include prostaglandins and leukotrienes, and for adhesion formation, the biochemicals include interleukins. The omega fatty acids of the compositions of the present invention inhibit the above-mentioned prostaglandin and leukotriene syntheses through interference with the cyclooxygenase and lipoxygenase pathways, respectively, and also inhibit interleukin production. Interleukin-2 is a potent mediator of the **inflammatory** response. Therapeutic measures that lower interleukin levels are associated with a decreased **inflammatory** response and often improved clinical outcome. Competitive inhibition by the omega fatty acids of the compositions of the present invention interferes with the utilization of arachidonic acid in both cyclooxygenase and lipoxygenase pathways, and renders the production of prostaglandins and leukotrienes largely inoperative. The same competitive inhibition principle applies to the diminution of interleukin production by the omega fatty acids of the compositions of the present invention.

Fatty acids are a class of organic compounds that are characterized by a long hydrocarbon chain terminating with a carboxylic acid group. Fatty acids have a carboxyl end and a methyl (i.e., "omega") end. **Omega-3 fatty acids** are derived from marine sources, while **omega-6 fatty acids** are derived from botanical sources. In addition to the difference in their origins, these omega fatty acids may be distinguished based on their structural characteristics.

Omega-3 fatty acids are a family of polyunsaturated fatty acids where the unsaturated carbon most distant from the carboxyl group is the third carbon from the methyl terminus. **Omega-3 fatty acids** have the following general formula: ##STR1## where R is a saturated or unsaturated, substituted or unsubstituted, branched or straight chain alkyl group having from 1 to 20 carbon atoms. Preferably, R is an

unsaturated straight chain alkyl having from 13 to 17 carbon atoms (i.e., an **omega-3 fatty acid** having from 18 to 22 total carbon atoms), and containing from 2 to 6 carbon-carbon double bonds. In a preferred embodiment, the compositions of the present invention comprise **omega-3 fatty acids** which contain 20 carbon atoms with 5 carbon-carbon double bonds, or 22 carbon atoms with 6 carbon-carbon double bonds, including (but not limited to) eicosapentaenoic acid ("EPA") and docosahexaenoic acid ("DHA"): ##STR2##

Similarly, **omega-6 fatty acids** are a family of unsaturated fatty acids where the unsaturated carbon most distant from the carboxyl group is the sixth carbon from the methyl terminus. **Omega-6 fatty acids** have the following general formula: ##STR3## where R is a saturated or unsaturated, substituted or unsubstituted, branched or straight chain alkyl group having from 1 to 20 carbon atoms. Preferably, R is an unsaturated straight chain alkyl having from 10 to 14 carbon atoms (i.e., an **omega-6 fatty acid** having from 18 to 22 total carbon atoms), and containing from 2 to 6 carbon-carbon double bonds. In a preferred embodiment, the compositions of the present invention comprise **omega-6 fatty acids** which contain 18 carbon atoms with 3 carbon-carbon double bonds, or 20 carbon atoms with 4 carbon-carbon double bonds, including (but not limited to) gamma-**linolenic acid** ("GLA") and dihomo-gamma-**linolenic acid** ("DHGLA"): ##STR4##

As mentioned above, the omega fatty acid compositions of the present invention may comprise **omega-3 fatty acids** or **omega-6 fatty acids** or a combination of omega-3 and **omega-6 fatty acids**. The omega fatty acids are present in the compositions in amounts sufficient to inhibit **inflammation** and adhesion formation in a patient when administered to a body cavity thereof. Moreover, a single omega fatty acid may be employed, or a mixture of two or more omega fatty acids may be used. For example, the compositions of the present invention may contain a single omega-3 or **omega-6 fatty acid**, two or more omega-3 or **omega-6 fatty acids**, an **omega-3 fatty acid** and one or more **omega-6 fatty acids**, an **omega-6 fatty acid** and one or more **omega-3 fatty acids**, or two or more **omega-3 fatty acids** and two or more **omega-6 fatty acids**.

In addition to omega fatty acids, the compositions of the present invention contain a nonionic surfactant. The nonionic surfactant of the composition facilitates the effective administration of the omega fatty acids to the tissues of the body cavity under treatment. The surfactant solubilizes the omega fatty acid and also acts as an emulsifying and/or dispersing agent which increases the permeability of the tissues toward the omega fatty acid of the composition. Preferred nonionic surfactants include polyethylene fatty acid esters and polysorbates.

The omega fatty acid compositions of the present invention may further comprise cyclooxygenase inhibitors and other additives and preservatives such as dextrose and vitamin A.

Cyclooxygenase inhibitors of the compositions of the present invention include any compound which effectively inhibits cyclooxygenase, including (but not limited to) acetylating and non-acetylating inhibitors. Cyclooxygenase inhibitors which acetylate cyclooxygenase

(i.e., "acetylating inhibitors") include acetylsalicylic acid (aspirin) and salicylsalicylic acid, as well as salts thereof. Cyclooxygenase inhibitors which do not acetylate cyclooxygenase (i.e., "non-acetylating inhibitors") include (but are not limited to) salicylates such as salicylic acid, trilisate, and diacid, and salts thereof. Other cyclooxygenase inhibitors include naproxen, piroxicam, indomethacin, sulindac, meclofenamate, diflunisal, tolmetin, phenylbutazone, ibuprofen, fenoprofen, ketoprofen and nabumetome.

The cyclooxygenase inhibitors are present in the composition in amounts sufficient to inhibit **inflammation** and adhesion formation in a patient when administered to a body cavity of the patient in combination with the omega fatty acid. A single cyclooxygenase inhibitor may be employed, or a mixture of two or more different inhibitors may be used.

The compositions of the present invention may also contain other additional optional ingredients including but not limited to, vitamin A and dextrose. These components provide composition stability and body cavity tissue permeability.

For purposes of administration, the compositions of the present invention may be formulated in any suitable manner for application to the tissues of the body cavity which are to be treated. Such formulations contain effective amounts of the omega fatty acid and nonionic surfactant, as well as one or more pharmaceutically acceptable carriers or diluents. More specifically, the formulations of the present invention may be administrated in the form of liquids containing acceptable diluents such as saline and sterile water, or may be administered as suspensions, emulsions or gels containing acceptable diluents or carriers to impart the desired texture, consistency, viscosity and appearance. Such acceptable diluents and carriers are familiar to those skilled in the art and include (but are not limited to) fatty alcohols, fatty acids, fatty esters, organic and inorganic bases, steroid esters, triglyceride esters, phospholipids such as lecithin and cephalin, polyhydric alcohol esters, hydrophobic lanolin derivatives, hydrocarbon oils, cocoa butter waxes, silicon oils, preserving agents, pH balancers and cellulose derivatives. One skilled in the art may further formulate the omega fatty acid and nonionic surfactant in an appropriate manner, and in accordance with accepted practices, such as those disclosed in Remington's Pharmaceutical Sciences, Gennaro, Ed., Mack Publishing Co., Easton, Pa. 1990 (which is incorporated herein by reference in its entirety).

As mentioned above, the omega fatty acid and nonionic surfactant are present in the composition in an amount sufficient to inhibit **inflammation** and adhesion formation in a patient when administered to a body cavity of the patient. When formulated for such administration, the omega fatty acid may be present in an amount ranging from 5% to 60% by weight (based on the total weight of the formulation), more preferably from 20% to 60% by weight, and most preferably from 35% to 60%. Similarly, the nonionic surfactant may be present in an amount ranging from 1% to 15%, more preferably from 2% to 10%, and most preferably from 3% to 5%.

Further optional ingredients include a cyclooxygenase inhibitor optionally present in an amount ranging from 0.5% to 1% by weight, vitamin A optionally present in an amount ranging from 1% to 2% by weight, and dextrose optionally present in an amount ranging from 2% to 4% by weight. Example 1 illustrates representative formulations of the compositions of the present invention.

The compositions of the present invention are administered to the tissues of the body cavity of a patient to inhibit **inflammation** and/or adhesion formation. As used herein, a body cavity is any space or potential space within the body. Examples of suitable body cavities to which the compositions may be administered include the following cavities: oral, abdominal, pleural, thoracic, pericardial, joint, rectal, bladder, and tympanic.

Accordingly, the compositions of the present invention may be used to treat active **inflammatory** conditions, including active diseases or more chronic, subacute diseases. For example, the compositions may be used in the treatment of both the early and late stages of **inflammatory** arthritis, **inflammatory** bowel disease, pericarditis, peritonitis, and pleuritis. The compositions may also be administered for dental applications. For example, the compositions are useful in preventing **inflammation** after tooth extraction or for treating various forms of gum disease. More specifically, after a periodontist performs gum surgery, an amount of the composition in liquid form may be applied directly to the wound, or may be used to bathe the inflamed tissues as a rinse. Alternatively, the composition may be applied daily in the form of a gel directly on the inflamed tissue for an effective period of time (such as one to two weeks following surgery). The compositions are also useful in inhibiting adhesion formation during intracavitary surgery and arthoscopy.

The compositions of this invention may be administered in liquid form by instillation into a body cavity directly at the time of surgery, thoracoscopy, laparoscopy, arthroscopy, cystoscopy, injection, or other procedure. The composition may also be applied directly to the lung, pericardium, synovium, tympanic membrane, or abdominal organs. Following application, the composition may be left to bathe the aforementioned organs, or removed by suction after having been lavaged within the cavity. Depending upon the anatomic site involved and the patient's clinical condition, additional applications may be employed.

The compositions of the present invention may be applied to a body cavity to inhibit **inflammation** under the following representative conditions. A patient with chronic synovitis of the knee may be injected or instilled with the composition into the knee three or four times a year. A patient with adhesive capsulitis of the shoulder would also benefit by administration of the composition after manipulation of the shoulder under anesthesia. A patient with chronic **inflammatory** lung disease may have the composition injected into the pleural space 6-12 times a year (depending upon the patient's clinical course). A patient with interstitial cystitis (chronic **inflammation** of the bladder) may have the composition administered via a cystoscope or urinary catheter 6-12 times a year. A patient with chronic **inflammatory** bowel disease which has an ulcerated and hemorrhagic intestinal mucosa may receive an annual lavage at the time of colonoscopy (e.g., the composition is dispersed through the entire colon as the colonoscope is slowly removed).

The compositions of the present invention may also be applied to a body cavity to inhibit adhesion formation under the following representative conditions. A patient undergoing laparotomy (surgery of the abdominal cavity) or operative laparoscopy (endoscopic surgery of the abdominal cavity) may receive a one-time lavage with the composition at the conclusion of the procedure to prevent adhesion formation. A woman patient plagued with chronic endometriosis may similarly be treated

according to the above method. At the time of operative laparoscopy (with or without laser ablation), a composition of this invention may be used to lavage the abdominal cavity to prevent adhesion formation.

DETD The following examples are provided for purposes of illustration, not limitation.

EXAMPLES

Example 1

Omega Fatty Acid Formulations

In this example, the formulations of various omega fatty compositions of the present invention are disclosed. In the following compositions, the **omega-3 fatty acids**, eicosapentaenoic acid and docosahexaenoic acid, are referred to as "EPA" and DHA," respectively, and the **omega-6 fatty acids**, gamma-**linolenic acid** and dihomo-gamma-**linolenic acid** are referred to as "GLA" and "DHGLA," respectively. The compositions may be formulated by mixing the following ingredients according to the weight percentages shown.

A. **Omega-3 Fatty Acid** Compositions

Formulation I:	
EPA	30-50%
DHA	5-10%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Acceptable carriers and/or diluents	30-60%
Formulation II:	
EPA	30-50%
DHA	5-10%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Salicylate	0.5-1%
Acceptable carriers and/or diluents	30-60%
Formulation III:	
EPA	30-50%
DHA	5-10%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Vitamin A	1-2%
Acceptable carriers and/or diluents	30-60%
Formulation IV:	
EPA	30-50%
DHA	5-10%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Salicylate	0.5-1%
Vitamin A	1-2%
Acceptable carriers and/or diluents	30-60%

Formulation V:

EPA	30-50%
DHA	5-10%
Polysorbate	2-8%
Salicylate	0.5-1%
Vitamin A	1-2%
Acceptable carriers and/or diluents	30-60%

Formulation VI:

EPA	30-50%
DHA	5-10%
Vitamin A	1-2%
Pectin and/or Gelatin	20-35%
Sodium Carboxymethylcelluose (Dispersed in a plasticized hydrocarbon gel composed of 5% polyethylene in mineral oil)	5-10%
Flavoring	0.5-1%
Acceptable carriers and/or diluents	30-60%

B. **Omega-6 Fatty Acid** Compositions

Formulation VII:

GLA and/or DHGLA	30-50%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Acceptable carriers and/or diluents	30-60%

Formulation VIII:

GLA and/or DHGLA	30-50%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Salicylate	0.5-1%
Acceptable carriers and/or diluents	30-60%

Formulation IX:

GLA and/or DHGLA	30-50%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Vitamin A	1-2%
Acceptable carriers and/or diluents	30-60%

Formulation X:

GLA and/or DHGLA	30-50%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Salicylate	0.5-1%
Vitamin A	1-2%
Acceptable carriers and/or diluents	30-60%

Formulation XI:

GLA and/or DHGLA	30-50%
Polysorbate	2-8%
Salicylate	0.5-1%
Vitamin A	1-2%
Acceptable carriers and/or diluents	30-60%

Formulation XII:

GLA and/or DHGLA	30-50%
Vitamin A	1-2%
Pectin and/or Gelatin	20-35%
Sodium Carboxymethylcelluose	

	5-10%
(Dispersed in a plasticized hydrocarbon gel composed of 5% polyethylene in mineral oil)	
Flavoring	0.5-1%
Acceptable carriers and/or diluents	30-60%

C. Omega-3 and **Omega-6 Fatty Acid** Compositions

Formulation XIII:	
EPA	15-25%
DHA	5-10%
GLA and/or DHGL	15-25%
Polyethylene fatty acid esters	2-8%
Dextrose	2-4%
Acceptable carriers and/or diluents	30-60%
Formulation XIV:	
EPA	15-25%
DHA	5-10%
GLA and/or DHGL	15-25%
Polysorbate	2-8%
Acceptable carriers and/or diluents	30-60%
Formulation XV:	
GLA and/or DHGLA	20-30%
EPA	10-20%
DHA	5-10%
Vitamin A	1-2%
Pectin and/or Gelatin	20-35%
Sodium Carboxymethylcelluose	5-10%
(Dispersed in a plasticized hydrocarbon gel composed of 5% polyethylene in mineral oil)	
Flavoring	0.5-1%
Acceptable carriers and/or diluents	30-60%
Formulation XVI:	
GLA and/or DHGLA	20-30%
EPA	10-20%
DHA	5-10%
Vitamin A	1-2%
Pectin and/or Gelatin	20-35%
Sodium Carboxymethylcelluose	5-10%
(Dispersed in a plasticized hydrocarbon gel composed of 5% polyethylene in mineral oil)	
Flavoring	0.5-1%
Acceptable carriers and/or diluents	30-60%

Example 2

Inhibition of **Inflammation**

Two patients with **inflammatory** joint disease are plagued with chronically painful, swollen knees. Both patients are taking several oral anti-**inflammatory** agents, but still suffer from active disease. For the first patient, 1 to 3 ml of a liquid formulation of a composition of this invention is injected directly into the joint cavity. For the second patient, 500-1000 ml of the same formulation is used to lavage the joint space. Both patients experience improved

symptoms, and the treatment is repeated every one to two months as needed.

Example 3

A 16-year-old girl who has active ulcerative colitis, a type of **inflammatory** bowel disease, is allergic to sulfa compounds (the commonly used drug to treat this condition is azulfidine). Prior to the present invention, the only other acceptable treatment plan would be high doses of corticosteroids. However, those compounds can produce serious systemic side effects, such as stunted growth, fluid retention, osteoporosis, increased risk of infection, and hypertension. Accordingly, a liquid formulation of a composition of this invention is administered in the form of a retention enema for 2 to 4 hours. This is repeated on a daily basis for two weeks, resulting in improved symptoms.

If significant symptoms persist, the formulation is applied directly through a colonoscope at the time of examination, thereby dispersing composition through the entire colon as the colonoscope is slowly removed.

Example 4

A patient with carcinoma of the colon undergoes a colon resection for removal of the tumor. The surgeon is concerned that post-operative adhesion formation might occur, thereby placing the patient at significant risk for developing a bowel obstruction. Therefore, the surgeon, prior to closing the abdominal cavity, instills directly into the abdomen, 1000 ml of a liquid formulation of a composition of this invention. This is allowed to bathe the tissue for a period of 5 minutes, after which time it is removed by suction, and the abdomen is then closed.

Example 5

A woman is undergoing operative laparoscopy (with or without laser for endometriosis). This condition results from islands of endometrial tissue that become implanted outside the uterine cavity within the abdomen. After ablation of this abnormal tissue at the time of surgery, the surgeon wishes to minimize adhesion formation (which is particularly important in the pelvic area, because adhesions here can in themselves produce significant pain and infertility). Five hundred to 1000 mls of a liquid formulation of a composition of this invention is introduced through the laparoscope into the abdominal cavity. This is allowed to bathe the tissue for a period of 5 minutes, after which time it is removed by suction prior to removal of the laparoscope and closure.

Example 6

Interstitial cystitis is a painful chronic **inflammatory** condition of the bladder of unknown cause for which current modes of therapy are not effective. Patients suffering from this condition are treated by the composition of this invention by instilling, either directly through a cystoscope or via foley catheter, 100 to 500 mls of a liquid formulation of the composition into the bladder cavity. The fluid remains in the bladder until it is expelled through urination, or drained by the surgeon. Treatment is then repeated every two to four weeks as needed.

From the foregoing, it will be appreciated that, although specific embodiments of this invention have been described herein for the purposes of illustration, various modifications may be made without

departing from the spirit and scope of the invention. Accordingly, the invention is not limited except by the appended claims.

CLM What is claimed is:

1. A method for inhibiting tissue adhesion in a body cavity of a warm-blooded animal, comprising administering to the body cavity an effective amount of a composition comprising an omega fatty acid, a nonionic surfactant, a cyclooxygenase inhibitor, and a pharmaceutically acceptable carrier or diluent.

2. The method of 1 wherein the body cavity is selected from the group consisting of oral, abdominal, pleural, thoracic, pericardial, joint, rectal, bladder, and tympanic cavities.

3. The method of claim 1 wherein the omega fatty acid is an **omega-3 fatty acid.**

4. The method of claim 1 wherein the omega fatty acid is an **omega-6 fatty acid.**

5. The method of claim 1 wherein the omega fatty acid is a mixture of an **omega-3 fatty acid** and an **omega-6 fatty acid.**

6. The method of claim 3 wherein the **omega-3 fatty acid** is selected from the group consisting of eicosapentaenoic acid, docosahexaenoic acid, and mixtures thereof.

7. The method of claim 4 wherein the **omega-6 fatty acid** is selected from the group consisting of gamma-**linolenic acid,** dihomo-gamma-**linolenic acid,** and mixtures thereof.

8. The method of claim 1 wherein the nonionic surfactant component a polyethylene fatty acid ester.

9. The method of claim 8 wherein the polyethylene fatty acid ester is polysorbate.

10. The method of claim 1 wherein the cyclooxygenase inhibitor is a salicylate.

11. The method of claim 1 wherein the composition further comprises vitamin A.

12. A method for inhibiting scar formation in a body cavity of warm-blooded animal, comprising administering to the body cavity an effective amount of a composition comprising an omega fatty acid, a nonionic surfactant, a cyclooxygenase inhibitor, and a pharmaceutically acceptable carrier or diluent.

13. The method of 12 wherein the body cavity is selected from the group consisting of oral, abdominal, pleural, thoracic, pericardial, joint, rectal, bladder, and tympanic cavities.

14. The method of claim 12 wherein the omega fatty acid is an **omega-3 fatty acid.**

15. The method of claim 12 wherein the omega fatty acid is an **omega-6 fatty acid.**

16. The method of claim 12 wherein the omega fatty acid is a mixture of an **omega-3 fatty acid** and an **omega-6 fatty acid.**

17. The method of claim 14 wherein the **omega-3 fatty acid** is selected from the group consisting of eicosapentaenoic acid, docosahexaenoic acid, and mixtures thereof.

18. The method of claim 15 wherein the **omega-6 fatty acid** is selected from the group consisting of gamma-**linolenic acid**, dihomo-gamma-**linolenic acid**, and mixtures thereof.

19. The method of claim 12 wherein the nonionic surfactant component a polyethylene fatty acid ester.

20. The method of claim 19 wherein the polyethylene fatty acid ester is polysorbate.

21. The method of claim 12 wherein the cyclooxygenase inhibitor is a salicylate.

22. The method of claim 12 wherein the composition further comprises vitamin A.

23. A method for inhibiting tissue adhesion in a body cavity of a warm-blooded animal, comprising administering to the body cavity an effective amount of a composition comprising an omega fatty acid, a nonionic surfactant, a cyclooxygenase inhibitor, vitamin A, and a pharmaceutically acceptable carrier or diluent.

24. A method for inhibiting scar formation in a body cavity of a warm-blooded animal, comprising administering to the body cavity an effective amount of a composition comprising an omega fatty acid, a nonionic surfactant, a cyclooxygenase inhibitor, vitamin A, and a pharmaceutically acceptable carrier or diluent.

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NCL NCLM: 514/560.000
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EXF 514/163; 514/549; 514/560; 514/725
ARTU 125
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 25 OF 33 USPATFULL
AN 95:22900 USPATFULL
TI Enteral formulations for treatment of **inflammation** and infection
IN Forse, R. Armour, Brookline, MA, United States
Chavali, Sambasiva, Boston, MA, United States
PA New England Deaconess Hospital Corporation, Boston, MA, United States (U.S. corporation)
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RLI Continuation-in-part of Ser. No. US 1994-201682, filed on 25 Feb 1994
DT Utility
FS Granted

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EXNAM Primary Examiner: Griffin, Ronald W.
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN No Drawings

AB The present invention features saponin containing enteral formulations for treatment of infection and **inflammation**. These saponin containing formulations are particularly useful in conjunction with oils
rich in .omega.3 polyunsaturated fatty acids such as fish oils and flax oil but also show benefits with .omega.6 rich oils such as borage oil, black currant seed oil, canola oil and rapeseed oil. These formulations may also contain a lignan from the sesamin family.

PARN REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of U.S. patent application Ser. No. 08/201,682, entitled "Anti-**inflammatory** and Infection Protective Effects of Sesamin-Based Lignans", filed Feb.

25, 1994, on an application of the presents inventors, the disclosure of which is incorporated herein by reference.

SUMM BACKGROUND OF THE INVENTION

The present invention relates to dietary manipulation for the treatment of disease. More particularly, the present invention relates to the use saponins in an enteral formulation for treatment of infection and **inflammation**.

The last decade has seen an explosion in the exploration of the interaction between diet and disease. In particular, the effects of various amino acids and lipids in the diet on a variety of conditions including heart disease, hypercatabolic states, liver disease, immunosupresssion, and infection treatment have been uncovered. Often, the effects are far removed from the norm and as such are unexpected. One of the most important developments of this type has been the discovery that by changing the dietary lipid content, positive effects in health treatment beyond plasma fat modification could be achieved. While the early work in modifying lipid content and type in diet came from an understanding that saturated fats cause particular problems in heart disease, later work determined that not just the use of polyunsaturated fats but also the type of polyunsaturated fat was important.

There are three major families of polyunsaturated fatty acids: .omega.3, .omega.6 and .omega.9. The names are based on location of the closest double bonds to the methyl end of the fatty acid; that is, if the closest double bond is between the third and fourth carbon atoms from the methyl group, the molecule is classified as an .**omega**.**3 fatty acid** while if the double bond is between the 6th and 7th carbon atoms, it is classified as an .**omega.6 fatty acid**. Mammals can desaturate or elongate fatty acid chains but cannot interconvert fatty acids from one family to another. The most important dietary fatty acids are the C.sub.18 and C.sub.20 fatty acids, primarily linoleic (C18:2.omega.6), **linolenic acid** (C18:3.omega.3), .gamma.-**linolenic acid** (C18:3.omega.6) and dihomo-.gamma.**linolenic acid** (C20:3.omega.6). Manipulation of the content of these fatty acids changes the ratio of arachidonic, eicosapentanoic, and decahexanoic acids (C20:4.omega.6, C20:5.omega.3, and C22:6.omega.receptively) and can cause far reaching effects in terms of immunosuppression, response to hypercatabolic states, and infection. For example, U.S. Pat. No. 4,752,618, issued Jun. 21, 1988 on an application of Mascioli et al., the disclosure of which is incorporated herein by reference, discloses the beneficial effects of .**omega.3 fatty acids** in the treatment of infection. In U.S. Pat. No. 5,260,336, issued Nov. 3, 1993 on an application of Forse et al., the disclosure of which is also incorporated herein by reference, concerns a method of minimizing the effect of catabolic illness or infection using an oil such as olive oil which is rich in .omega.9 fatty acids. Other similar patents and articles, such as U.S. Pat. No. 4,810,726, issued. Mar. 7, 1989 on an application of Bistrian et al., the disclosure of which is also incorporated herein by reference, disclose other means of treating illness using fatty acid dietary manipulation.

The "culprit" in many diets appears to be the high level of .**omega.6 fatty acids**, primarily linoleic acid, a precursor for the formation of arachidonic acid which is a substrate for the production of pro **inflammatory** dienoic

eicosanoids including PGE.sub.2 and TxA.sub.2 which can lead to elevated levels of thromboxane A.sub.2 and related prostanoids. Elevation of these prostanoids has been linked to problems in response to endotoxin challenge and other infection states. Accordingly, the new wave in diets has been to minimize the **.omega.6 fatty acid** content (which, although an essential fatty acid, is not needed in the quantities found in most commercial oils) while maximizing the **.omega.3 fatty acids** (e.g., fish oil) and .omega.9 fatty acids (e.g., olive oil). Similarly, although sesame oil has long been promoted as having medicinal benefits, it is only recently that the effects have been traced to sesamin (and its related lignans) in the sesame oil. In fact, U.S. patent application Ser. No. 08/201,682, filed Feb. 25, 1994, on an application of the same inventors, discloses that sesamin can promote resistance to infection and reduce **inflammation**. Thus, materials which modify lipid content in the diet may have important and surprising health effects.

The present invention uses saponins to treat infection and reduce **inflammation**. It has also been found that these saponins can work in concert with other agents such as fish oils to provide quicker (and consequently better) protection against infection.

Saponins are surface active triterpene or sterol glycosides. Although the saponins are found mainly in plants, they have also been found in certain marine animals such as echinoderms like starfish and sea cucumbers. Most saponins are non-toxic when taken orally, but many are toxic upon i.m. or i.v. injection. Saponins are most often ingested by man in legumes such as chick peas and soy beans. In fact, it has been theorized that legumes rich in saponins may reduce the threat of heart disease based, in part, on the finding that saponins can reduce plasma cholesterol levels in animals. See, e.g., Newman et al., Poultry Science 37 42-45(1957).

However, the main medicinal use for saponins appears to be their properties as immunostimulating substances or adjuvants. Reports of immunopotentiating advantages using saponins go back over fifty years (see, e.g., Thibault and Richou, C.R. Soc. Biol. 121 718-721 (1936)). While saponins are available from many sources, much of the work on immunostimulation has used saponins derived from the inner bark of the South American soaptree, Quillaja saponaria Molina. These saponins, normally designated as the Quill A saponins, remain the principal medicinal saponins in use today.

Although many other medicinal uses have been hypothesized for saponins, there has been no systematic proof that any effects other than use as an adjuvant is medicinally feasible. However, saponins have been found in some plants used in traditional or folk remedies. For example, saponins are present in ginseng which has long been used in Asia for treatment of a variety of conditions. Similarly, other homeopathic remedies also may contain saponins. The recent interest in homeopathic remedies has lead to a further exploration of the properties of materials such as saponins.

Accordingly, an object of the invention is to provide an enteral dietary supplement containing saponins.

Another object of the invention is to provide a means of treating

infection and/or **inflammation** using saponins.

A further object of the invention is to provide a dietary supplement useful in improving the effects of .**omega.3 fatty acids** on treatment of infection.

An additional object of the invention is to provide a dietary supplement useful in improving the uptake of polyunsaturated fatty acids (e.g., EPA and DHA) in tissue.

A still further object of the invention is to provide a method of treating infection and/or **inflammation** using dietary manipulation.

These and other objects and features of the invention will be apparent from the following description and the claims.

SUMMARY OF THE INVENTION

The present invention features enteral formulations for treatment of **inflammation** and infections, as well as methods of treatment itself. These formulations are based on the surprising properties of saponins, a material that is often used as an adjuvant but not as the medicament itself. The saponins are effective with standard enteral formulations such as safflower oil dietary supplements and appear to have additive, or even synergistic, effects with .**omega.3 fatty acid** formulations such as those derived from fish oil or linseed oil. The saponins can also be used with sesamin and related lignans from sesame oil to provide particularly advantageous diets. These saponins could also be included in other food products such as margarines and butter as well as dietary supplements. Such other food products and dietary supplements are included in the enteral formulations herein.

More particularly, the present invention features an enteral formulation adapted for treatment of infection or **inflammation** in a patient which includes an effective amount of a saponin as an active ingredient. The term "effective amount" means a sufficient amount of the saponin to cause the clinical effect in terms of anti-**inflammation** and/or anti-infection properties. This effective amount can vary due to a number of factors including type of saponin and personal metabolism. For Quill A, one of the most readily available saponins, this effective amount appears to be about 0.1% -1.0% by weight of the enteral diet, with a 0.25% amount being preferred. For other saponins, with different purification and potency, different effective amounts may easily be determined.

The enteral formulation useful in the invention may include particular fatty acids or other materials which have similar anti-**inflammatory** properties. For example, the previously cited U.S. Pat. No. 4,752,618 discloses that .**omega.3 fatty acids** may have anti-infection properties. An enteral formulation which includes these .**omega.3 fatty acids** in conjunction with the saponins is, therefore, advantageous. Preferred sources of .omega.3 are the fish oils, and linseed (flax) oil, most preferably the oils derived from cold water fish which have at least 10% of their lipid content in .**omega.3 fatty acids** and flax oil

which contains approximately 55% **linolenic acid** (18:3 .omega.3). Examples of the useful cold water fish include menhaden and sardine. In fact, as is shown later in the examples, the addition of saponins to an enteral formulation containing **.omega.3 fatty acids** causes less lagtime until the beneficial effects of the **.omega.3 fatty acids** occur and increased uptake of **.omega.3 fatty acids** into tissue. These saponins may also yield beneficial effects with other dietary oils such as borage oil, black currant seed oil, canola oil, and rapeseed oil.

Another additive useful in an enteral formulation is a lignan of the sesamin family. Previously cited U.S. patent application Ser. No. 08/201,682 discloses the anti-infection and anti-**inflammatory** properties of these lignans. The lignans preferred include sesamin, episesamin, sesaminol, espisemsaminol, and sesamolin. A combination therapy including these lignans and the saponins may be particularly advantageous.

Any enteral formulation preferably includes essential amino acids, essential fatty acids, and/or essential vitamins and minerals. The enteral formulations of the present invention may be in the form of a dietary supplement or used as a total enteral feeding regimen. If the later, these essential nutrients are required while even in a supplement, the addition insures that the patient is obtaining these nutrients.

The enteral formulation such as is previously described are particularly useful in treating infection and **inflammation**. In fact, these formulations may be used in at risk patients to prevent possible infection or **inflammation**. Further, when used with the other formulations such as the **.omega.3 fatty acids**, the time to effective action may be reduced.

The following description and non-limiting examples further elucidate the invention.

DETAILED DESCRIPTION

The present invention provides an enteral formulation useful in treating **inflammation** and/or infection. This enteral formulation includes an effective amount of a saponin such as Quill A, possible in conjunction with a diet rich in **.omega.3 fatty acids** or a diet containing a lignan such as sesamin. As such, saponins show remarkable promise as additives in treating infection states, particularly acute infections e.g., sepsis.

DETD The following examples, which all use saponins in enteral diets, further explain the invention.

EXAMPLE 1

This example explains the procedure used to create the diets used for test purposes. The two diets basic diets were made, a safflower oil diet (SO) which had large quantities of **.omega.6 fatty acids**, primarily in the form of linoleic acid, and a fish oil (FO) diet which had a large percentage of **.omega .3 fatty acids**. The oil portion of the safflower oil diet was made by taking 52 g of safflower oil (SVO

Specialty Products, Culberton, Mont.) and mixing it with 88 g of palm oil and 10 g of Trisum, a high oleic sunflower oil. The fish oil diet used menhaden oil, which has 32% .omega.3 polyunsaturated fats, primarily in the form of eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), as the fish oil. The fish oil portion of the fish oil diet was made by blending 8 g safflower oil, 125 g of fish oil, 35 g of palm oil and 10 g of Trisum. These physical mixtures of oils were prepared to maintain the saturated, monounsaturated, and polyunsaturated fat contents identical in both experimental diets. However, the polyunsaturated fatty acids in the former is .omega.6 type and in the latter is .omega.3. One hundred fifty grams of each oil mixture was added to 850 g of AIN-76, a fat-free basal diet which contained essential minerals and vitamins. For each 1000 g of either enteral diet, 15% by weight was in form of fat with the fat calories being approximately 30% of the total (as recommended by the Surgeon General).

The combination of the fat and the AIN-76 fat-free basal diet had 0.05% t-butyl hydroxy tolulene added as an antioxidant, and the diets were stored in individual daily rations, flushed with nitrogen to minimize oxidation, at 4.degree. C. The animals were fed ad libium every day before dusk.

Separate groups of Balb/c mice were maintained on the safflower oil diet, the fish oil diet, and the two diets supplemented with saponins. Plasma was sampled at 4, 7 and 10 days and the fatty acid compositions of phospholipids in the plasma were determined by gas chromatography following a thin layer of chromatography.

The relative mole percent of individual fatty acids (including linoleic acid and arachidonic acid) incorporated into the plasma phospholipids and the tissues were determined. There was substantially no difference in the fatty acid pattern for the safflower oil diet vs. the safflower oil with saponin diet but the fish oil diet vs. fish oil with saponin diet was another matter. At day 4, the relative percentages of eicosapentanoid acid and decahexenoic acid (DHA) were twice as high in the plasma phospholipids of mice consuming the fish oil with saponins diet as compared with the fish oil alone. By day 7, the differences disappeared. However, the levels of tissue polyunsaturated .**omega.3 fatty acids** increased at day 7 and remained elevated until day 10.

EXAMPLE 2

In this example, Balb/C mice were maintained ad libium on one of the diets described in Example 1, the safflower oil diet, for three weeks. Safflower oil diets are commonly used for enteral nutrition. A first group received just the safflower oil diet (SO) while the second group had the safflower oil diet supplemented with 0.25% saponins (SO+). There were twenty animals in the first group and seventeen in the second group.

At the end of three weeks, all the animals in both groups underwent cecal ligation and puncture. To perform this procedure, the mice were anaesthetized and then shaved over the anterior abdominal wall. A midline incision, approximately 2 cm long, was made, sufficient to expose the cecum and adjacent intestine. With a 3-0 silk suture, the cecum was tightly ligated at its base without causing bowel obstruction.

The cecum was then punctured twice with a 22 gauge needle, gently squeezed to exude feces and to insure that the two puncture holes did not close. The abdominal incision was then closed and 1 ml of saline was administered subcutaneously for fluid resuscitation. This cecal ligation

and puncture is a widely accepted form of infection model to resemble abdominal sepsis. See, e.g., C. Baker et al., "Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model," Surgery (August 1983), pp. 331-335. Survival of the mice is the normal measure of treatment effectiveness.

In addition, ten animals were fed each diet to serve as controls and were a sham operated; this means, that the abdominal operation was performed but cecal ligation and puncture was not carried out.

TABLE 1

Diets	24 hours	48 hours	72 hours	96 hours
SAFFLOWER OIL (SO)	20 (100)	14 (70)	6 (30)*	4 (20)*
SAFFLOWER OIL + SAPONINS (SO+)	17 (100)	16 (94)	15 (88)**	15 (88)**

Table 1 shows the survival on the SO diet vs. the SO+ diet. While all the animals in each group were alive at 24 hours, the number of animals alive at 48, 72 and 96 hours decreases rapidly for the safflower oil group while the group being treated with the safflower plus saponin diet shows very little mortality. The first number is the number of animals remaining alive while the second is a percent remaining alive. At 72 hours, the number of animals surviving is statistically significant ($p<0.05$ using a student t test) while at 96 hours, the data are even better ($p<0.01$). The groups of animals consuming the diets supplemented with saponins showed no mortality.

Accordingly, this shows that adding the saponins to a safflower oil diet has significant anti-infection effects.

EXAMPLE 3

The beneficial effects of feeding diets enriched with safflower oil (15 wt %=30% total calories) supplemented with or without saponins (0.25%) was tested in an infection model. Groups of 10 female Balb/c mice, 6-8 weeks old, were fed the two diets for 3 weeks. The plasma levels of thrombxane B.sub.2 (TBX2), tumor necrosis factor (TNF)-.alpha. and other proinflammatory mediators were determined in plasma 90 minutes after an interperitonial injection of lipopolysacchride (LPS) (20mg/kg).

TABLE 2

	SAFFLOWER OIL (SO)	SO + SAPONINS
TXB.sub.2 (pg/ml*)	466 .+-. 98	257 .+-. 48#
TNF-.alpha. (pg/ml*)	380 .+-. 100	100 .+-. 40#

*means .+-. S.D of determinations following LPS i.p injection in mice (n 10 in each group.)
#p < 0.05.

The increase in survival of animals in Example 2 were associated with significantly lower concentrations (45%) of the LPS-induced TBX.sub.2 and TNF-.alpha. in the circulation while the AA content, a 5precursor for the formation of dienoic eicosanoids (such as TBX.sub.2), was unchanged for the groups of mice fed safflower oil diets containing saponins as described in Example 1. These data suggest that saponins possess anti-**inflammatory** properties which may include inhibiting the activities of phospholipase A.sub.2 or cyclooxygenase enzymes. Further, the ability of saponins to markedly lower (74%) the LPS-induced in vivo production of TNF-.alpha. suggests a possible mechanism by which dietary saponins confer protection against infection irrespective of the type of polyunsaturated fatty acid in the diet. These data indicate that inclusion of saponins in an enteral formulation containing different types of polyunsaturated fatty acids (.omega.3, .omega.6, or .omega.9) could benefit critically ill patients.

EXAMPLE 4

In this experiment, Balb/c mice were again maintained on either the safflower oil diet alone or the safflower oil diet supplemented with 0.25% of the saponin Quill A. Spleens were isolated aseptically at 1, 2 and 3 weeks and single cell suspensions were prepared. One million spleen cells were stimulated with either concanavalin A (Con A-1 mg/ml) or lyopopolysacchride (lps-10 .mu.g/ml) for twenty-four hours, both of which are known to induce the production of proinflammatory mediators. Cell free supernatants were collected and the amounts of prostaglandin E.sub.2 (PGE2) were determined by immunoassay. The PGE.sub.2 levels in the supernatants from the spleen cells of the animals treated with the saponins were significantly lower (see Table 2) than those with the safflower oil diet alone on day 7 ($p<0.05$). After two or three weeks of feeding, the mean concentrations of the PGE.sub.2 were not significantly different. These data suggest the saponins exhibited anti-**inflammatory** properties and that feeding safflower oil diets with saponins may have selected a cell population which participated in defending the host against infection.

TABLE 3

	Con A	LPS
SAFFLOWER OIL (SO)	114 .+-. 20	248 .+-. 32
SAFFLOWER OIL + SAPONINS (SO+)	70 .+-. 13	153 .+-. 11

All valves in pg/ml at day 7.

Since it is known that fish oil diets will provide anti-infection properties, the ability of the saponin addition to provide a more rapid incorporation of **.omega.3 fatty acids** in the fatty acid profiles of the phospholipids in the plasma and in the tissues suggest that this may speed the action of the fish oil. If so, this effect may be important in treating infection, particularly with post-operative patients.

The foregoing examples are merely exemplary and one skilled in the art may determine other enteral diets and methods of treatment using such an enteral diet which falls within the scope of the present invention. The invention is defined not by these examples but rather by the following claims.

CLM What is claimed is:

1. An enteral formulation for the treatment of infection or **inflammation** in a patient having as an active ingredient an effective amount of Quil A saponin, said enteral formulation further comprising a source of dietary polyunsaturated fatty acids, said fatty acids forming at least significant part of the fat content given to said patient.

2. The enteral formulation of claim 1 wherein said source of dietary polyunsaturated fatty acids is selected from the group consisting of fish oils and vegetable oils rich in **.omega.3 fatty acids.**

3. The enteral formulation of claim 2 wherein said source of **.omega.3 fatty acids** is a fish oil selected from the group consisting of fish oils having at least 10% of the lipid content as **.omega.3 fatty acids.**

4. The enteral formulation of claim 1 further comprising a lignan selected from the group consisting of sesamin, episesamin, sesaminol, episesaminol, and sesamolin.

5. The enteral formulation of claim 4 wherein said lignan is added to said enteral formulation in the form of sesame oil.

6. The enteral formulation of claim 4 wherein said lignan is added to said enteral formulation in the form of purified lignan.

7. The enteral formulation of claim 1 :further comprising essential amino acids.

8. The enteral formulation of claim 1 further comprising essential vitamins and minerals.

9. The enteral formulation of claim 1 wherein said enteral formulation includes a dietary oil selected from the group consisting of borage oil, black currant seed oil, canola oil and rapeseed oil.

10. A method of treating infection and preventing infection in at risk persons comprising the step of enteral administration thereto of an effective amount of an enteral formulation in conjunction with a source of dietary polyunsaturated fatty acids which constitute at least a significant part of the fat fed to such persons, said enteral formulation having as its active ingredient Quil A saponin.

11. The method of claim 10 wherein said source of dietary polyunsaturated fatty acids is selected from the group consisting of fish oils and vegetable oils rich in **.omega.3 fatty acids.**

12. The method of claim 11 wherein said source of **.omega.3 fatty acids** is a fish oil selected from the group of fish oils having at least 10% of its lipid content as **.omega.3 fatty acids.**

13. The method of claim 10 wherein said administration step comprises administration of a lignan selected from the group consisting of sesamin, episesamin, sesaminol, episesaminol, and sesamolin in conjunction with said enteral formulation.

14. The method of claim 13 wherein said lignan is added to said enteral formulation in the form of sesame oil.

15. The method of claim 13 wherein said lignan is added to said enteral formulation in the form of a purified lignan.

16. The method of claim 10 wherein said administration step comprises administration of an oil selected from the group consisting of borage oil, black currant seed oil, canola oil, and rapeseed oil in conjunction with said saponin.

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INCLS: 424/195.100; 424/DIG.013; 426/804.000; 426/810.000; 514/464.000; 514/468.000; 514/783.000; 514/825.000; 514/886.000; 514/887.000; 514/904.000; 514/905.000
NCL NCLM: 514/198.000
NCLS: 424/755.000; 424/764.000; 424/765.000; 424/776.000; 424/DIG.013; 426/804.000; 426/810.000; 514/464.000; 514/468.000; 514/783.000; 514/825.000; 514/886.000; 514/887.000; 514/904.000; 514/905.000
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EXF 424/195.1; 424/DIG.13; 514/198; 514/464; 514/468; 514/783; 514/825; 514/886; 514/887; 514/904; 514/905; 426/804; 426/810
ARTU 185
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 26 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 4
AN 95105782 EMBASE
DN 1995105782
TI Alpha-**linolenic acid** in the treatment of rheumatoid arthritis: A double blind, placebo-controlled and randomized study: Flaxseed vs safflower seed.
AU Nordstrom D.C.E.; Honkanen V.E.A.; Nasu Y.; Antila E.; Friman C.; Konttinen Y.T.
CS Helsinki Univ. Central Hospital, Division of Rheumatology, Unioninkatu 38,FIN-00170, Helsinki, Finland
SO Rheumatology International, (1995) 14/6 (231-234).
ISSN: 0172-8172 CODEN: RHINDE
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DT Journal; Article
FS 026 Immunology, Serology and Transplantation
031 Arthritis and Rheumatism
037 Drug Literature Index
038 Adverse Reactions Titles
LA English
SL English
AB In rheumatoid arthritis various pro-**inflammatory** metabolites of arachidonic acid (AA), such as leukotriene B4 (LTB4) and prostaglandin E2 (PGE2), contribute to tissue destruction and pain. In contrast to AA, which is an **omega-6 fatty acid**, the **omega-3 fatty acids**, after having been liberated from the cell membrane phospholipids, are further converted into the non- or anti-**inflammatory** eicosanoids LTB5 and PGI3. AA concentration is an important regulatory step in the synthesis of both prostanoids and leukotriens. Dietary supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has therefore been used to decrease the ratio of AA to EPA or DHA to obtain beneficial clinical effects. EPA and DHA are found in animal fat and are quite expensive compared to their precursor alpha-**linolenic acid** (alpha-LNA) found in **flaxseed oil**. We, therefore, performed a placebo-controlled trial with alpha-LNA in 22 patients with rheumatoid arthritis, using a linoleic acid preparation as a placebo. After a 3-month follow-up, the treatment group showed an increased bleeding time, but the clinical, subjective (global assessment, classification of functional status, joint score index, visual analogue scale, pain tendereness score) and laboratory parameters (haemoglobin,

erythrocyte sedimentation rate, C-reactive protein) did not show any statistical alterations. AA, EPA and DHA did not change either in spite of a significant increase in alpha-LNA in the treatment group. Thus, 3-month's supplementation with alpha-LNA did not prove to be beneficial in rheumatoid arthritis.

CT Medical Descriptors:
*rheumatoid arthritis: DT, drug therapy
adult
arachidonic acid metabolism
article
bleeding time
clinical article
clinical trial
diarrhea: SI, side effect
diet supplementation
double blind procedure
erythrocyte sedimentation rate
female
human
intramuscular drug administration
joint function
male
oral drug administration
pain assessment
priority journal
randomized controlled trial
Drug Descriptors:
***linolenic acid: CT, clinical trial**
***linolenic acid: AD, drug administration**
***linolenic acid: DT, drug therapy**
***linolenic acid: AE, adverse drug reaction**
*linseed oil: AE, adverse drug reaction
*linseed oil: CT, clinical trial
*linseed oil: AD, drug administration
*linseed oil: DT, drug therapy
*safflower oil: AE, adverse drug reaction
*safflower oil: DT, drug therapy
*safflower oil: AD, drug administration
*safflower oil: CT, clinical trial
azathioprine: DT, drug therapy
corticosteroid: DT, drug therapy
corticosteroid: AD, drug administration
docosahexaenoic acid
fatty acid
gold: DT, drug therapy
gold: AD, drug administration
hydroxychloroquine: DT, drug therapy
icosapentaenoic acid
leukotriene b4: EC, endogenous compound
linoleic acid
membrane phospholipid: EC, endogenous compound
methotrexate: DT, drug therapy
nonsteroid antiinflammatory agent: DT, drug therapy
penicillamine: DT, drug therapy
placebo
prostaglandin e2: EC, endogenous compound
prostanoid: EC, endogenous compound
salazosulfapyridine: DT, drug therapy

RN (**linolenic acid**) 1955-33-5, 463-40-1; (linseed oil) 8001-26-1; (safflower oil) 8001-23-8; (azathioprine) 446-86-6; (docosahexaenoic acid) 25167-62-8, 32839-18-2; (gold) 7440-57-5; (hydroxychloroquine) 118-42-3, 525-31-5; (icosapentaenoic acid) 25378-27-2, 32839-30-8; (leukotriene b4) 71160-24-2; (linoleic acid) 1509-85-9, 2197-37-7, 60-33-3, 822-17-3; (methotrexate) 15475-56-6,

59-05-2, 7413-34-5; (penicillamine) 2219-30-9, 52-67-5; (prostaglandin e2)
363-24-6; (salazosulfapyridine) 599-79-1

L13 ANSWER 27 OF 33 USPATFULL
AN 93:93826 USPATFULL
TI Monounsaturated fat as dietary supplement to minimize the effects of catabolic illness
IN Forse, R. Armour, Brookline, MA, United States
Mascioli, Edward A., Needham, MA, United States
PA New England Deaconess Hospital Corporation, Boston, MA, United States (U.S. corporation)
PI US 5260336 19931109 <--
AI US 1992-876189 19920430 (7)
DT Utility
FS Granted

REP	US 4196218	Apr 1980	514/560.000	Thiele
	US 4528197	Jul 1985	514/560.000	Blackburn
	US 4752618	Jun 1988	514/549.000	Mascioli et al.
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REN Martindale, The Extra Pharmacopoeia (1982) 28th edition, p. 697.
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Sagher, F. A. et al., J. Pediatr. Gastroenterol. Nutr. 13: 83-89 (1991).
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Kelley, V. E. et al., Transplantation 48(1): 98-102 (Jul. 1989).
EXNAM Primary Examiner: Waddell, Frederick E.; Assistant Examiner: Henley, III, Raymond J.
CLMN Number of Claims: 24
ECL Exemplary Claim: 1
DRWN No Drawings
AB Disclosed is a method of minimizing the effects of a catabolic illness in an individual by administering to the individual a diet which is controlled in the type of fatty acid intake. The diet comprises an oil rich in .omega.9 monounsaturated fatty acids, preferably oleic acid. Oils rich in monounsaturated fatty acids include olive oil, canola oil and high oleic acid safflower or high oleic acid sunflower oil. The diet
can also be administered to an individual to minimize infection or to minimize the risk of infection in the individual. A dietary supplement useful in methods of the invention and a structured lipid are also disclosed.

SUMM BACKGROUND OF THE INVENTION

Under normal nutritional and physiological conditions, fuel requirements
of the body are met primarily by glucose and fatty acid metabolism. However, during abnormal metabolic stress states induced by trauma or sepsis, one of the effects is a decrease of fat and glucose utilization.
Under these conditions, a high rate of bodily protein catabolism occurs.
This metabolic response results in the acceleration of protein degradation and an elevation of energy expenditure, or hypercatabolism. Bodily protein catabolism provides the precursors for oxidation of branched chain amino acids and the synthesis and release of alanine for hepatic metabolism as a gluconeogenic substrate. Urinary nitrogen

excretion is often elevated and the individual suffers a negative nitrogen balance. If the stress is persistent, the nitrogen losses will eventually deplete the individual's protein stores resulting in a progressive deterioration of lean body mass and multiple organ failure.

Stress of injury in an individual, such as trauma or sepsis, is often accompanied by total or partial dysfunction of the gastro-intestinal tract. These individuals are often hospitalized and must receive most or all of their daily nutritional requirements parenterally and/or enterally in order to sustain protein synthesis and avoid malnutrition. For example, many patients are administered total parenteral nutrition which includes a source of fatty acids. Standard parenteral nutrition diets have long chain fatty acid triglyceride (LCT) emulsions, composed of either soybean or safflower oil, as the primary lipid source. These oils are high in **.omega.6 fatty acids**, particularly linoleic acid.

As an alternative or additive fatty acid source, medium chain triglycerides (MCT) formed from saturated fatty acids with 6-12 carbon backbones have been used in various formulations. MCTs are metabolized more rapidly than long chain triglycerides in that they enter the body through the portal rather than lymphatic pathway. Metabolic products of MCT do not require carnitine to enter into the mitochondria where they undergo .beta.-oxidation.

One current high protein enteral liquid nutrition supplement, REPLETE.TM. (Clintec Nutrition Company), provides MCTs to minimize diarrhea caused by fat intolerance. Another formulation for enteral feedings, IMMUN-AID.TM. (Kendall McGaw Laboratories, Inc.), includes both MCTs and canola oil as sources of fat and is reported to improve immune function in an immunocompromised, stressed patient. Canola oil is discussed in the IMMUN-AID.TM. literature as being included in the composition as a source of **.omega.3 fatty acids**, which are reported to improve the cell-mediated immune response in animal models.

Canola oil not only provides **.omega.-3 fatty acids**; it and olive oil are the richest natural sources of monounsaturated fatty acids. Another new enteral product, PROMOTE.TM. (Ross Laboratories), uses high oleic acid safflower oil, canola oil and MCT's as lipid sources. The primary monounsaturated fatty acid in canola oil, olive oil, and high oleic acid safflower oil is oleic acid, an .omega.9 monounsaturated fatty acid. Monounsaturated oils are not appreciably elongated to a 20-carbon fatty acid. Thus, unlike oils high in polyunsaturated fatty acids, such as .omega.3 and **.omega.6 fatty acids**, administration of monounsaturated oils can not act as substrate in the prostanoid synthesis pathway which forms prostaglandins from fatty acids. Since the amount of certain prostaglandins in the system, particularly elevated levels of the "2" series prostaglandins, have been shown to be related to deleterious response to endotoxin in animal studies, reducing primarily with oils has positive health effects.

Accordingly, an object of the invention is to provide a method of treating patients having metabolic stress or sepsis using a diet which includes modifications in fatty acid content.

Another object of the invention is to provide an enteral or parenteral solution which can assist a patient combat challenge by metabolic stress or sepsis.

A further object of the invention is to provide an enteral or parenteral diet having reduced polyunsaturated fat content without the problems caused by high levels of MCT'S.

These and other objects and features of the invention will be apparent from the detailed description.

SUMMARY OF THE INVENTION

The present invention features a method of minimizing the effects of catabolic illness or response to sepsis in an individual. The method comprises administering a diet, preferably a parenteral diet, which is controlled in the type of fatty acid intake to the individual. The diet of the present invention includes an oil having an .omega.9 monounsaturated fatty acid as its major fatty acid component as the primary lipid source. The preferred .omega.9 monounsaturated fatty acid is oleic acid. Oils rich (about 45-85%) in oleic acid include olive oil, canola oil and high oleic acid safflower or high oleic acid sunflower oil.

Individuals to be treated using this high monounsaturated fat diet are catabolic for a variety of reasons; for example, the catabolism may be due to surgery, burns, trauma or **inflammatory** process or the individuals may have an infection at the time of administration of the diet or may be at high risk of infection due to some immunocompromise. Individuals at risk of infection include those suffering with secondary immunosuppression due to chemotherapy or diabetes mellitus, protein-malnourished patients, or patients undergoing surgery, e.g., abdominal or thoracic surgery. While the oil rich in monounsaturated fatty acids can be administered enterally or parenterally, parenteral administration is preferable because of better absorption by the body. Often patients in stressed states have difficulty absorbing any food, let alone long chain fatty acids. The .omega.9 monounsaturated fatty acids can constitute 15-85% of the total fatty acids in diet, providing 5-75% of the total calories.

The .omega.9 monounsaturated fats can be given in a variety of ways. For example, the diet could include just the high oleic acid oils or there could be a physical mixture of the .omega.9 monounsaturated fat containing oils with other fat sources, such as medium chain triglycerides or fish oils. These other oils may be necessary to provide essential fatty acids. In the alternative, the .omega.9 monounsaturated oils could be administered as part of a structured lipid. This structured lipid could have the form: ##STR1##

where at least one of the R.sub.1, R.sub.2 and R.sub.3 is an monounsaturated fat, and the others of R.sub.1, R.sub.2 and R.sub.3 are selected from the group consisting of C.sub.6 -C.sub.12 saturated fats, C.sub.14 -C.sub.20 primarily unsaturated fats, and mixtures thereof. Preferred structured lipids would contain one C.sub.6 -C.sub.12 fat, one .omega.9 monounsaturated fat (e.g., oleic acid), and one C.sub.14 -C.sub.20 unsaturated fat (e.g., linoleic or **linolenic acid**).

The invention also features a dietary supplement, preferably a parenteral dietary supplement, having 10 to 90 percent by weight of an oil having an .omega.9 monounsaturated fatty acid as the major fatty acid component, 1-2 percent by weight of an emulsifier and sterile water. The dietary supplement may also include an osmolality modifier and other essential nutrients. The dietary supplement can be administered to an individual to minimize the effects of a catabolic

illness or an infection in the individual or to minimize the risk of infection in the individual.

DETD DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method, diet, and dietary supplement to minimize the effects of catabolic illness. The method utilizes dietary control of the type of fatty acids provided to an individual experiencing catabolic stress following surgery, trauma or burn injury. A diet useful in the method includes as the primary lipid source an oil having a monounsaturated fatty acid as the major fatty acid component is administered to the individual. Although the diet can be administered enterally, parenteral administration is preferred. Olive oil and canola oil, which have oleic acid, a monounsaturated fatty acid, as the major fatty acid component, are preferred sources of the oil for administration to a stressed individual. The diet provides improved support of the individual's physiology during a catabolic illness (e.g., decreased metabolic acidosis, decreased hypotension, and improved maintenance of metabolic rate). Individuals to be treated may be catabolic due to trauma, burn, AIDS, sepsis, cancer or surgery.

A diet including an oil having an .omega.9 monounsaturated fatty acid as the major fatty acid component can also be administered to an individual to minimize the effects of infection in the individual or to minimize the risk of subsequent infection in an individual at risk of infection. Individuals at risk of infection include those suffering with secondary immunosuppression due to chemotherapy or diabetes mellitus, protein-malnourished individuals, or individuals undergoing abdominal surgery. The infections can be wound infections, empyemas, bacteremias, abscesses, or septicemias. These infections are caused by a variety of infectious agents including bacteria (e.g., E. coli, Pseudomonas, Klebsiella, Staphylococcus aureus or albus), viruses (e.g., Herpes simplex or zoster), parasites, and fungi (e.g., Candida).

Conventional dietary supplements have primarily soybean or safflower oil as their lipid or fatty acid source. By replacing these predominantly .**omega.6 fatty acid**-containing oils with monounsaturated fatty acid-containing oils, the effects of a catabolic illness or the effects of infection or risk of infection in an individual may be reduced. The oil included in the diet of the present invention typically comprises 45-85% .omega.9 monounsaturated fatty acids and comprises 5-75% of the total calories of the diet. The monounsaturated fatty acids preferably comprise 15-85% of the total fatty acids in the diet.

Preferred oils useful in treating stressed individuals are olive oil and canola oil which are rich in oleic acid. High oleic acid safflower or high oleic acid sunflower oils may also be used. Olive oil is primarily a mixture of mono-and-triglycerides, which are esters of glycerol with fatty acids. Olive oil also contains small quantities of free fatty acids, glycerol, phosphatides, pigments, carbohydrates, sterols and resinous substances. The major fatty acids present as glycerides in olive oil are oleic (18:1), linoleic (18:2), palmitoleic (16:1), palmitic (16:0), and stearic (18:0). The three principal fatty acids are typically present in the following ranges: oleic acid 56-83%, palmitic acid 7-20%, and linoleic acid 3-20%. Canola oil typically comprises 62-83% oleic acid and 10% **.omega.3 fatty**

acids. High oleic acid safflower oils and high oleic acid sunflower oils can have 60-80% .omega.9 monounsaturated fatty acids and have primarily .omega.6 (e.g., linoleic) fatty acids as the remaining oils. The oils may be concentrated to provide a high percentage of monounsaturated fatty acids per unit volume.

The oils having .omega.9 monounsaturated fatty acids as the major fatty acid component can be administered to the individual enterally or parenterally. When the oil is administered parenterally, it is normally in the form of an emulsion of 1-40% lipid in water. The emulsion can be combined with other nutrients to provide a final concentration of monounsaturated fatty acid of 1-30%.

The invention also features a dietary supplement, preferably for parenteral use, having 10 to 90% by weight of an oil having an .omega.9 monounsaturated fatty acid as the major fatty acid component, 1-2% by weight of an emulsifier and sterile water. Higher levels (25-75%) of the monounsaturated fat are preferred. Emulsifiers useful in the supplement include egg yolk phospholipids and soybean phospholipids. The dietary supplement may also include 1-3% of an osmolality modifier such as glycerin. Such dietary supplements can be administered to an individual to minimize the effects of a catabolic illness in the individual or minimize the effects of infection or subsequent infection in an individual at risk of infection.

The following non-limiting examples will show the efficacy of the present invention.

EXAMPLE 1

This Example illustrates that animals fed a diet in which the primary lipid sources are oils rich in .omega.9 monounsaturated fatty acids rather than a diet containing oils rich in **.omega.6 fatty acids** minimizes the effects of endotoxin shock upon challenge with endotoxin. The following fats were used in this study: fish or menhaden oil (FI), an oil rich in **.omega.3 fatty acids**; safflower oil (SA), an oil rich in **.omega.6 fatty acids**; black currant seed oil (BC), an oil rich in **.omega.6 fatty acids** (linoleic plus gamma **linolenic acid**); medium chain triglycerides (MCT), primarily saturated C.sub.8 and C.sub.10 fatty acids; and olive oil, an oil rich in .omega.9 monounsaturated fatty acids. To compare the specific effects of each lipid to modulate the response to endotoxin shock, male Sprague Dawley rats (300 grams) were fed either one of the above fats in a semipurified diet or standard chow (CH) for 7 weeks.

At the end of the feeding period, venous catheters were placed for continuous endotoxin administration for 8 hours (3 mg/kg/hr). The endotoxin was a lipopolysaccharide derived from E. coli (Difco Laboratories). Arterial catheters were also inserted for hemodynamic monitoring and blood gas sampling. Resting energy expenditure (REE) was calculated by indirect calorimetry at 0, 2, 4, 6 and 8 hours. REE was calculated from oxygen consumption and C0.sub.2 production using the Weir equation.

The diets are standard rat chow and semipurified diets such that the fat source is controlled and specific to that dietary group. The oil content is raised in the semipurified diets (except the standard rat chow) so the diet contains 13 percent by weight of lipid as opposed to the normal

5.5 percent. This allows 27 percent of the dietary calories to be lipid-derived as compared with the standard 14 percent. All the diets were equicaloric, except for the standard chow which contained less fat and the MCT diet which contains slightly less calories per gram.

The results demonstrate that animals fed olive oil developed the least acidosis as manifested by two factors: pH at Hour 7 (pH 7.49; $p<0.04$) and serum HCO.sub.3 at Hour 8 (16mEq/l; $p<0.008$). Since acidosis often accompanies protein catabolism, reducing acidosis should correlate with lower levels of protein catabolism. In contrast, animals fed black currant seed oil, standard chow or MCT, each demonstrated a pH at Hour 7 of 7.45, the fish oil diet had a pH of 7.47, and the safflower diet had a pH of 7.46. In addition, serum HCO.sub.3 at Hour 8 in animals fed black currant seed oil or MCT was 13 mEq/l while the values for fish oil, safflower oil and standard chow were 15, 14 and 15 mEq/l, respectively. This result also indicates reduced catabolism by diet modification.

Another important finding was in the resting energy expenditure (REE). Maintenance of the REE shows minimalizing of protein catabolism and better host response to the catabolic insult. REE was best preserved in the group of animals fed olive oil (Hour 6, 125 Kcal/kg/hr olive oil vs. 108 Kcal/kg/hr for standard chow, $p<0.03$). Accordingly, the olive oil diet provided significant advantages in treating endotoxin shock.

EXAMPLE 2

This Example illustrates one procedure for forming a dietary supplement for minimizing the effects of a catabolic illness in an individual or for enhancing resistance to infection.

The dietary supplement is preferably in the form of an oil emulsion. For each liter of emulsion, 50-400 grams of refined and bleached oil rich in .omega.9 monounsaturated fatty acids is mixed with about 11 grams of an emulsifier, e.g., egg yolk phospholipids USP, 22.5 grams of an osmolality modifier, e.g., glycerin USP, and sterile water USP to bring the volume to a liter. Specifically, the oil is added to a high shear mixer such as a Waring mixer with steel blades operated at 1,600 RPM. The phospholipids are added slowly to the oil and mixed at high speed for 6 minutes. Eight hundred milliliters of sterile water is added in a steady stream to the phospholipid and oil mixture and emulsified for 20 minutes at 1600 RPM. The attainment of the oil-in-water emulsion is confirmed by the "drop dispersion test." Emulsification is continued until the coarse oil emulsion disperses freely in water but not in oil.

The coarse emulsion is then passed through a high speed homogenizer five times until particle size is less than 1 micron. At that time, five more passes through the high speed homogenizer are performed and with each pass, glycerin is added to the emulsion. During the last five passes, additional water is added to make the final emulsion volume up to the one liter batch. Normally, all volumes are multiplied ten-fold and a ten liter batch is mixed at once.

Aliquots of the emulsion are set aside for measuring particle size which should be between 0.24 and 0.75 microns. The solutions are then passed through a five micron particle filter into sterile and pyrogen free evacuated containers. The emulsion is then sterilized at low temperature

(105.degree. C.) for 25 minutes. The solutions are cooled to room temperature and stored in the dark at 9.degree. C. for one week. Prior to patient administration, the samples are retested for particle size and the presence of bacterial or endotoxin contamination. If the particle size is greater than 1 micron or the endotoxin concentration is greater than 1 ng, the batch of emulsion is discarded.

While the method and dietary supplement disclosed herein will not necessarily prevent catabolism in an individual or prevent the onset of infection, it will minimize the effects of a catabolic illness and promote survival of infected patients. The specific method and dietary supplement set forth herein are illustrative and those skilled in the art may determine other modifications and variations of these procedures. Such other modifications and variations are included within the scope of the following claims.

CLM What is claimed is:

1. A method of minimizing the effects of a catabolic illness in an individual, comprising administering to the individual a diet which is controlled in the type of fatty acid intake, said diet including as the primary lipid source an oil having a .omega.9 monounsaturated fatty acid as the major fatty acid component.

2. The method of claim 1 wherein said monounsaturated fatty acid is oleic acid.

3. The method of claim 1 wherein said oil is selected from a group consisting of olive oil, canola oil and high oleic acid safflower or high oleic acid sunflower oil.

4. The method of claim 1 wherein said oil comprises 45-85% .omega.9 monounsaturated fatty acid.

5. The method of claim 1 wherein said monounsaturated fatty acid comprises 15-85% of the total fatty acids in the diet.

6. The method of claim 1 wherein said oil comprises 5-75% of the total calories of the diet administered to the individual.

7. The method of claim 4 wherein said oil is administered enterally.

8. The method of claim 5 wherein said fatty acids are administered enterally.

9. The method of claim 1 wherein said oil is administered parenterally as an emulsion of 1-40% lipid in water.

10. A method of minimizing the effects of an infection in an individual and minimizing the effects of subsequent infection in an individual at risk of infection, comprising administering to the individual a diet which is controlled in the type of fatty acid intake, said diet including as the primary lipid source an oil having a .omega.9 monounsaturated fatty acid as the major fatty acid component.

11. The method of claim 10 wherein said monounsaturated fatty acid is oleic acid.

12. The method of claim 10 wherein said oil is selected from a group consisting of olive oil, canola oil and high oleic acid safflower or high oleic acid sunflower oil.

13. The method of claim 10 wherein said oil comprises 45-85% monounsaturated fatty acid.

14. The method of claim 10 wherein said monounsaturated fatty acid comprises 15-85% of the total fatty acids in the diet.

15. The method of claim 10 wherein said oil comprises 5-75% of the total calories of the diet administered to the individual.

16. The method of claim 14 wherein said oil is administered enterally.

17. The method of claim 15 wherein said fatty acids are administered enterally.

18. The method of claim 10 wherein said oil is administered parenterally as an emulsion of 1-40% lipid in water.

19. The method of claim 10 wherein said patients are infected with infections selected from a group consisting of wound infections, empyemas, bacteremias, abscesses, and septicemias.

20. The method of claim 10 wherein said infections are caused by infectious agents selected from a group consisting of bacteria, viruses, parasites, and fungi.

21. The method of claim 10 wherein said individuals are at risk of infection at time of administration of the diet.

22. The method of claim 21 wherein said individuals at risk of infection are selected from a group consisting of individuals with secondary immunosuppression due to chemotherapy or diabetes mellitus, protein-malnourished individuals, and individuals undergoing surgery.

23. A method of minimizing the effects of a catabolic illness in an individual, comprising administering to the individual a dietary supplement including at least 5-40% by weight of an oil having an .omega.9 monounsaturated fatty acid as the major fatty acid component; 1-2% by weight emulsifier; and sterile water.

24. A method of minimizing the effects of an infection in an individual and minimizing the effects of subsequent infection in an individual at risk of infection, comprising administering to the individual a dietary supplement including at least 5-40% by weight of an oil having an .omega.9 monounsaturated fatty acid as the major fatty acid component; 1-2% by weight emulsifier; and sterile water.

INCL INCLM: 514/560.000
INCLS: 514/549.000
NCL NCLM: 514/560.000
NCLS: 514/549.000
IC [5]
ICM: A61K031-20
ICS: A61K031-22
EXF 514/549; 514/560; 514/552
ARTU 125
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 28 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 92325610 EMBASE
DN 1992325610
TI [Essential fatty acids: State of art].
ESSENTIELLE FETTSAUREN: STATE OF ART.
AU Singer P.
CS Melibokusstrasse 14,6140 Bensheim 3, Germany
SO Pharmazeutische Zeitung, (1992) 137/41 (86-88).

ISSN: 0031-7136 CODEN: PZSED5
CY Germany
DT Journal; (Short Survey)
FS 029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LA German
CT Medical Descriptors:
*lipid metabolism
antiinflammatory activity
drug activity
drug research
human
lipid blood level
membrane
nonhuman
nutrient
short survey
thrombocyte aggregation
Drug Descriptors:
*essential fatty acid: PD, pharmacology
*essential fatty acid: EC, endogenous compound
*fatty acid: EC, endogenous compound
*fatty acid: PD, pharmacology
*fish oil: PD, pharmacology
*icosanoid: EC, endogenous compound
*icosanoid: PD, pharmacology
*phospholipid: EC, endogenous compound
*phospholipid: PD, pharmacology
cyclosporin a: PD, pharmacology
docosahexaenoic acid: PD, pharmacology
icosapentaenoic acid: PD, pharmacology
linolenic acid: PD, pharmacology
omega 3 fatty acid: PD, pharmacology
omega 6 fatty acid: PD, pharmacology
RN (essential fatty acid) 11006-87-4; (fish oil) 8016-13-5; (cyclosporin a) 59865-13-3, 63798-73-2; (docosahexaenoic acid) 25167-62-8, 32839-18-2; (icosapentaenoic acid) 25378-27-2, 32839-30-8; (**linolenic acid**) 1955-33-5, 463-40-1
CN Maxepa

L13 ANSWER 29 OF 33 USPATFULL
AN 91:86762 USPATFULL
TI Method for reducing blood pressure levels in hypertensive persons
IN Sears, Barry D., Swampscott, MA, United States
PA BioSyn, Inc., Marblehead, MA, United States (U.S. corporation)
PI US 5059622 19911022 <--
AI US 1990-539384 19900618 (7)
RLI Division of Ser. No. US 1989-400288, filed on 29 Aug 1989, now abandoned
which is a continuation-in-part of Ser. No. US 1988-251139, filed on 28 Sep 1988, now abandoned
DT Utility
FS Granted
REP US 4681896 Jul 1987 514/560.000 Horrobin
US 4920098 Apr 1990 514/558.000 Cotter et al.
EXNAM Primary Examiner: Waddell, Frederick E.; Assistant Examiner: Henley, III, Raymond J.
LREP Crowley, Richard P.
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
AB The modulation of prostaglandin levels can be realized through the dietary intake of specified ratios of activated Omega 6 essentially fatty acids when combined with the appropriate amount of eicosapentaenoic acid (EPA), an **Omega 3**

fatty acid. The modulation of prostaglandins levels can be determined by changes in physiological parameters which are related to prostaglandin levels in mammals. Certain ratios of activated Omega 6 essentially fatty acids and EPA can have significant physiological benefits, whereas other ratios demonstrate detrimental physiological effects in mammals.

PARN REFERENCE TO PRIOR APPLICATIONS

This application is a divisional application of Ser. No. 07/400,288, filed Aug. 29, 1989, now abandoned which application is a continuation-in-part of Ser. No. 07/251,139, filed Sept. 28, 1988, now abandoned.

SUMM FIELD OF THE INVENTION

The present invention relates to both food products and pharmaceutical compositions containing specified activated Omega 6 essential fatty acids, gamma **linolenic acid** (GLA) and/or dihomo gamma **linolenic acid** (DGLA) combined with eicosapentaenoic acid (EPA), for the modulation of prostaglandin levels in mammals. Certain ratios of activated Omega 6 essential fatty acids and EPA successfully modulate the levels of beneficial prostaglandins that can provide effective treatment for existing disease states, or can be used as a prophylactic approach to prevent the onset of disease states such as cardiovascular disease and immune disorders. On the other hand, other ratios of the same fatty acids have a deleterious effect on existing disease conditions by increasing the levels of detrimental prostaglandins, and would be considered counterproductive in the treatment or prevention of disease states.

BACKGROUND OF THE INVENTION

Prostaglandins are a group of hormone like substances which are known to play a significant factor in virtually all body function. In particular, prostaglandins play important roles in controlling the cardiovascular and immunological systems of the human body. Yet as important are prostaglandins for human health, their production is totally dependent on the dietary intake of a specialized group of fatty acids known as essential fatty acids. Essential fatty acids cannot be made by the human body, and must be supplied in the diet to provide sufficient precursors from which to synthesize prostaglandins. The primary essential fatty acids belong to the Omega 6 family of essential fatty acids. The complexity and dynamics of the transformation of these Omega 6 essential fatty acids into prostaglandins is shown in FIG. 1.

The complexity of Omega 6 essential fatty acid metabolism, and thus the determination of which prostaglandins are produced, is due to the activity of various enzymes responsible for the biological transformation of these essential fatty acids. Differences in the enzyme activity control the relative levels of the true prostaglandin precursors: dihomo gamma **linolenic acid** (DGLA) and arachidonic acid (AA). The prostaglandins of the one series derived from DGLA are beneficial for the cardiovascular system, stimulate the immune system, and control hormone synthesis and release. On the other hand, the prostaglandins of the two series derived from AA can inhibit cardiovascular function, depress the immune system, and generally have diameterically opposed physiological functions to prostaglandins of the

one series. To maintain proper body function, both series one and two prostaglandins must be formed. Therefore, it is the balance of DGLA to AA in each body's cell that eventually determines the exact ratio of the one and two series prostaglandins that are formed. An overabundance of either the one or two series prostaglandins is not consistent with optimal physiological performance. The ratio of DGLA to AA is ultimately determined by the two primary enzymes that control the ratio of DGLA and AA in each cell. These two enzymes are delta-6 desaturase (D6D) and delta-5 desaturase (D5D).

The enzymes D6D and D5D are the rate controlling factors which determine the amounts of each of the prostaglandin precursors which will ultimately give rise to one series prostaglandins or two series prostaglandins. This becomes a primary factor for the treatment and possible prevention of cardiovascular disorders as the prostaglandins derived from AA (especially thromboxane A.sub.2) are considered to be the primary cause of cardiovascular disease.sup.1, whereas prostaglandins derived from DGLA (especially PGE.sub.1) are considered to be important in reducing the probability of developing cardiovascular disease..sup.2 Likewise, certain prostaglandins derived from AA (such as PGE.sub.2 and thromboxane A.sub.2) suppress the immune system, while prostaglandins from DGLA (such as PGE.sub.1) stimulate the immune system. Prostaglandins are also be formed from eicosapentaenoic acid (EPA). However, compared to the powerful physiological actions of prostaglandins of the one and two series, those derived from EPA are relatively neuter in theirphysiological actions.

Because EPA is a prevelant constituent in certain diets such as the Greenland Eskimos, and since the Greenland Eskimos have a very low incidence of cardiovascular disease, it has been assumed that EPA can treat or provide prophylaxis against various aspects of cardiovascular disease. This has been disclosed in British Patent Nos. 1,604,554 and 2,033,745.

Likewise, prior art has recognized the beneficial effects of GLA and/or DGLA as a treatment for cardiovascular disease in German Patent No. 2,749,492 and even earlier prior art concerning GLA in British Patent No. 1,082,624.

The use of a combination of many **Omega 6 fatty acids** with EPA and other **Omega 3 fatty acids** (i.e. docosahexanoic acid or DHA) which are not direct precursors for prostaglandin synthesis was disclosed in U.S. Pat. No. 4,526,902. However, this particular patent teaches that the roles of EPA and another fatty acid docsahexaenoic acid (DHA) are to compete with AA for various enzymes (i.e. cyclooxygenase) important in the production of prostaglandins, thereby leading to the inhibition of series two prostaglandins. This art does not refer to the critical role of EPA alone in its inhibitory action of the D5D enzyme. This prior art also makes the assertion that all **Omega 6 fatty acids** such as linoleic acid, GLA, and DGLA are biologically equivalent in terms of producing prostaglandins of the one series, and in particular PGE.sub.1.

It has been shown that in human adipose tissue (the primary storage site of fat) the ratio of linoleic acid to GLA to DGLA is approximately 100:2:1..sup.3 The reason for such a radically altered ratio of **Omega 6 fatty acids** is their

increased biological potency beyond the enzymatic step catalyzed by the enzyme delta-6-desaturase in the conversion of linoleic acid into GLA (see FIG. 1). As an example, it has been estimated that GLA was 163 times more effective in lowering cholesterol levels in humans than an equivalent dose of linoleic acid..sup.4 Likewise, studies in primates have indicated that DGLA has twice the biological potency of GLA in terms of reducing platelet aggregation which is mediated by the formation of PGE.sub.1..sup.5 Furthermore, there is strong evidence that with increasing age the body's ability to produce GLA from linoleic acid is highly compromised therefore making the assertion of the equivalence of all **Omega 6 fatty acids** even more unlikely..sup.6,7 These results of biological potency corresponds well with the actual levels of **Omega 6 fatty acids** found in humans. What this means is that to assume that all **Omega 6 fatty acids** have the same biological potency in terms of prostaglandin production would be to grossly skew the balance of GLA and DGLA to EPA required to optimize prostaglandin levels of the one and two series prostaglandins.

U.S. Pat. No. 4,681,896 has taught that combinations of activated **Omega 6 fatty acids** with combinations of **Omega 3 fatty acids** are useful in the treatment of atopic disorders. Although this patent discloses "that the presence of n-3 fatty acids in a combination will lead to some inhibition of the conversion of DGLA to arachidonic acid by the delta-5-desaturase", the patent does not disclose that certain weight combinations of activated **Omega 6 fatty acids** and **Omega 3 fatty acids** (i.e. EPA) would be benefical, whereas other weight combinations are harmful to humans. Likewise, it is not taught in the general literature that certain weight combinations of activated Omega 6 essential fatty acids (such as GLA or DGLA) in combination with EPA would be beneficial, whereas other weight combinations would be detrimental.

The reason why prior art has not discovered this critical aspect of essential fatty metabolism is that no long term human studies have been conducted with combinations of activated Omega 6 essential fatty acids and EPA. This is important since the conversion of DGLA to AA in humans is relatively slow, yet proceeds continuously..sup.8 This slow conversion of DGLA into AA can be exceptionally harmful. The studies described in this patent illustrates deficiencies in the prior art.

SUMMARY OF THE INVENTION

It is the object of this invention to eliminate the above discussed deficiencies in the prior art and to improve upon the prior art.

It is also an object of this invention to demonstrate that activated Omega 6 essential fatty acids in combination with EPA composition create superior therapeutic benefits and thus a superior prophylatic composition compared to prior art in the form of pharmaceutical formulations or as food products.

The basis of this invention is the use of the appropriate EPA weight amount in relation to the amount of activated Omega 6 essential fatty acids to control or modulate the rate of transformation of DGLA into AA.

Although the rate of transformation of DGLA in AA is relatively slow in man compared to other animal species.sup.8, the long term benefits of supplementation with GLA or DGLA as disclosed in prior art(Brit. Patent No. 1,082,624) would be highly diminished as the increased levels of GLA

or DGLA would simply eventually increase the levels of AA and thus increase the levels of prostaglandins of the two series being formed (see FIG. 1). Since the goal of this invention is to reduce such levels of prostaglandins derived from AA, while simultaneously increasing the production of prostaglandins from DGLA, the prior art concerning supplementation with GLA and DGLA alone would be counterproductive to the present invention.

EPA, but not other **Omega 3 fatty acids** (such as DHA) will inhibit this transformation of DGLA into AA in rats..sup.9 This has been shown in animals experiments using fish oil which contained both EPA and DHA and vegetable oil containing GLA. The GLA is readily metabolized into DGLA. However, the futher metabolism of DGLA and its transformation into AA was reduced. This reduction was only correlated with the amount of EPA, and there was no correlation with the presence of DHA..sup.9 This result is in accord with EPA acting as an inhibitor of D5D, whereas DHA does not. This in not taught in the prior art (U.S. Pat. No. 4,526,902) in which levels of DHA are considered important to that invention. The inhibitory effect of EPA on D5D by reducing the transformation of DGLA into AA will thereby increase the levels of precursors of the one series prostaglandins and simultaneously reduce the levels of the two series of prostaglandins. In this respect, the preferred combination of GLA and/or DGLA with EPA will have a much more selective effect on the modulation of prostaglandin levels than the prior art. Also, the preferred combinations of GLA and/or DGLA with EPA when compared to commonly used pharmaceuticals such as aspirin, corticosteroids, and anti-**inflammatory** drugs, such as ibuprofen and others, whose mode of action is to modulate prostaglandin levels by inhibiting the formation of all prostaglandins including the beneficial prostaglandins from the one series. The present invention will have a more selective benefit on the modulation of prostaglandin levels. Furthermore, preferred combinations of GLA and/or DGLA when combined with EPA will be more effective than disclosed in the above mentioned prior art. In fact, those combinations outside the weight ratios in this invention are detrimental to the health of mammals.

I have found that using a composition of GLA and/or DGLA in combination with EPA, provides substantial relief of existing cardiovascular and immune conditions. The preferred weight ratio of GLA and/or DGLA to EPA in the present invention is preferably 1:8. While these ratios are the preferred ratios, the ratio of GLA and/or DGLA to EPA may vary from 1:2 to 1:40.

The preferred physical form of the GLA, DGLA, and EPA would be as triglycerides, although other acceptable forms would include methyl or ethyl esters, monoglycerides, free fatty acids, or the appropriate salts of free fatty acids.

The preferred route of administration for the invention as a pharmaceutical would be orally as a capsule or tablet, although other routes of administration such as parenteral (intravenous, subcutaneous, and intramuscular), rectal, vaginal, buccal, and transdermal are feasible if the invention is formulated in such a manner to be successfully absorbed and utilized. As an example for parenteral administration, the perferred form would be as an emulsion with the knowledge that the ingredients of the emulsion must be physiologically compatible.

Given the critical importance of precise ratios of GLA and/or DGLA and EPA, as part of a mammal's dietary intake, on can also incorporate the invention in food products such as cooking oils, salad dressings, dairy products, emulsions, margarines, mayonnasie, and other foods which can accomodate such fatty acids. Furthermore, microencapsulation, using standard technology, can produce a granulated version of the invention. With such granulated versions, it is also possible to introduce the invention in an even wider variety of food products in which such granulated powders can be incorporated.

If triglycerides are used, then they must meet the basic technical specifications set by the World Health Organization in the Codex Alimentarius..sup.10 A further requirement for the EPA component is that it should be as low in cholesterol content as possible, and be free of high levels of excessive levels or Vitamins A and D. The decreased levels of Vitamins A and D eliminate the possibility of potentially toxic amounts of Vitamins A and D given with the invention. The reasons for the low cholesterol levels are two fold. First is the need for the reduced intake of dietary cholesterol which would be contraindicated for cardiovascular treatment and prophylaxis. The second reason is that the removal of cholesterol from EPA source also removes other contaminants commonly found in EPA sources such as PCB's. It has been shown that traditional methods of vegetable oil refining do not remove PCB's..sup.11 Furthermore, the use of high temperatures and high vacuum to remove pesticides and herbicides from vegetable oils, will cause extensive isomerization of the double bonds of EPA thereby rendering it ineffective as an inhibitor for D5D. Therefore, if one is using triglycerides containing EPA, the preferred final refining technique before inclusion into the invention will be the removal of PCB's without isomerization of the double bonds. This can be accomplished through the use of molecular distillation, supercritical fluid extraction, or other such techniques skilled to those in the art.

DESCRIPTION OF THE INVENTION

The invention consists of a defined combination of essential fatty acids containing GLA and/or DGLA with the appropriate weight amount of EPA to modulate precursor pools for prostaglandin production in mammals. Therefore, to fully describe the invention one must illustrate general methods of GLA, DGLA, and EPA preparation.

GLA in the triglyceride form can be most easily extracted and refined from vegetable seed sources using standard technology common in the edible oil industry to create an oil suitable for human consumption as defined by international standards..sup.11 Common sources of GLA would include borage, black current, evening primrose seeds and oat bran. Certain microorganisms can also be fermented to produce GLA in the triglyceride form which can likewise be refined to meet international standards established for an edible oil.

GLA isolated in the triglyceride form can be chemically or biochemically transformed into free fatty acids, salts of free fatty acids, methyl or ethyl esters, or monoglycerides which can be further fractionated by standard techniques into fractions with higher GLA content than found in the starting oils. Finally, GLA can be chemically synthesized by standard chemical techniques.

A good natural source DGLA does not exit, so that to make this essential

fatty acid, one must either elongate the free fatty acid, methyl or ethyl esters of GLA using standard techniques such as the malonic ester synthesis or to chemically synthesize the compound.

Like GLA, EPA can be easily extracted from natural sources such as plankton, krill, or marine animals. Also like GLA, EPA can be fermented under controlled conditions. In both cases, the extracted oil should be refined to meet all international standards for edible oils. Again like GLA, the triglyceride form of EPA can be altered either by chemical or biochemical means to produce free fatty acids, salts of free fatty acids, methyl or ethyl esters, or monoglycerides which can be further fractionated to give higher EPA contents than the starting oil in the triglyceride form. EPA can also be chemically synthesized.

For the purpose of illustration only, the invention will be described in connection with the method of preparation in various pharmaceutical and food products and its use in the treatment in certain cardiovascular and immune disorders and hence by extension, its use in the prophylaxis of such cardiovascular and immune disorders. However, it is recognized that various changes and modifications to the illustrated examples can be made by those persons skilled in the art, all falling within the spirit and scope of the invention.

DRWD FIG. 1 describes the biochemical relationships of GLA and EPA that is important in the modulation of prostaglandins. It is the effect of EPA as an inhibitior of the enzyme delta 5 desaturase that diverts the flow of GLA into dihomo gamma **linolenic acid** (DGLA) instead of its further metabolism into arachidonic acid (AA). As shown in the examples of the invention, the ratio of EPA to GLA is critically important in the successful modulation of prostaglandins.

DETD DESCRIPTION OF THE EMBODIMENTS

Example 1

Six hypertensive subjects with an average blood pressure of 150/92 were placed on a daily intake of 80 mg. of GLA and 640 mg. of EPA per day for 6 weeks. At the end of six weeks, their blood pressure was measured and was lowered to an average of 134/78. Their intake was then changed to 80 mg. GLA and 320 mg. EPA per day for another 2 weeks. At the end of this period, their blood pressure was measured and the average was found to be 140/83. Their intake was then changed to 80 mg. GLA and 160 mg. EPA per day for another 2 weeks. At the end of this 2 week period, their blood pressure was measured, and the average blood pressure was 146/88. Their intake was then changed to 80 mg. GLA and 80 mg. EPA for another 2 weeks. At the end of this period, their blood pressure was measured, and the average blood pressure reading was 156/95. Their intake was changed to 80 mg. GLA and 40 mg. EPA per day for another another 2 weeks. At the end of 2 weeks, their blood pressure was measured, and found to average 162/100. At this point they were switched back to 80 mg. GLA and 640 mg. EPA for a final 2 weeks. The average blood pressure was reduced to 143/85. The results concerning the cardiovascular effects of different GLA and EPA ratios are shown in Table 1.

TABLE 1

Effect of combinations of activated Omega 6 essential fatty acids and EPA on blood pressure in hypertensive individuals

Weeks	Ratio of EPA to GLA	Average Blood Pressure
Start	None	150/92
6	8:1	134/78
2	4:1	140/83
2	2:1	146/88
2	1:1	156/95
2	0.5:1	162/100
2	8:1	143/85

These results show that some ratios of EPA and GLA are beneficial in the treatment of existing hypertension, whereas other ratios are detrimental to the existing disease condition.

Example 2

36 healthy adults were split into 6 groups of 6 individuals. They were given the following amounts of dietary supplements for 6 weeks on a daily basis.

Group 1: 80 mg. GLA and 3200 mg. EPA.

Group 2: 80 mg. GLA and 1600 mg. EPA.

Group 3: 80 mg. GLA and 800 mg. EPA.

Group 4: 80 mg. GLA and 400 mg. EPA.

Group 5: 80 mg. GLA and 80 mg. EPA.

Group 6: 80 mg. GLA and 40 mg. EPA.

At the end of 6 weeks, each individual was asked to evaluate their energy levels, digestive system responses, and skin condition based on the various levels of supplementation on a subjective scale comparing the initial starting point to the final point at the end of the study.

The following questions were asked, and the individuals were asked to respond using a grading system ranging from +2 (significantly improved), +1 (somewhat improved), 0 (no change), -1 (somewhat worse), -2 (significantly worse).

Question 1. Where your energy levels altered during the study?

Question 2. Did your stool composition change during the study?

Question 3. Was your skin condition altered during the study?

Each of these questions was used to assess physiological function which is closely related to prostaglandin formation, and therefore serves as an indication as to how dietary supplementation with activated Omega 6 essential fatty acids and EPA could effect prostaglandin formation, and therefore utlimately physiological function.

Energy levels are related to fatigue. Prostaglandins derived from AA (such as thromboxane A.sub.2) are powerful vasoconstrictors that restrict the size of the capillary bed, and ultimately reduce the transfer rate of oxygen to muscle tissue. This lack of oxygen transfer will increase the levels of lactic acid in the muscle which causes

fatigue. On the other hand, prostaglandins derived from DGLA (such as PGE.sub.1) are powerful vasodilators which will increase the size of the capillary bed, thereby increasing oxygen transfer to muscle cells. However, if too much PGE.sub.1 is formed, the kidneys will undergo a corresponding increase in vasodilation resulting in increased urination, and subsequent electrolyte depletion. The electrolyte depletion will contribution to fatigue, thereby decreasing energy levels. Therefore, at either extreme of the DGLA to AA ratio in the cardiovascular system, fatigue is the end result. However, at the optimal balance of these two fatty acids, energy levels should increase. Therefore, the levels of DGLA to AA in the cardiovascular system will determine which prostaglandins are eventually produced, and thus ultimately determine the energy level of the individual.

Likewise, stool composition is a good indicator of prostaglandin formation in the gastrointensintal tract. Vasoconstrictors such as thromboxane A.sub.2 derived from AA will decrease the flow of water into the colon causing constipation. On the other hand, vasodilators such as PGE.sub.1 derived from DGLA will increase the flow of water into the colon. If too much AA is formed by dietary supplementation with activated Omega 6 essential fatty acids and an insufficient amount of EPA, then an individual will develop constipation. On the other hand, if too much DGLA is being formed, then an individual will develop diarrhea.

Again the appropriate balance of DGLA to AA is reflected in the stool composition. Thus the ratio of DGLA to AA in the mucosa that lines the gastrointensinal tract will determine which prostaglandins are ultimately produced which are reflected in the stool composition.

Finally, the skin also responds to changes in the DGLA to AA ratio which will manifest itself in the modulation of prostaglandins. Prostaglandins such as PGE.sub.2 derived from AA are pro-**inflammatory** and will increase existing skin disorders such as eczema or produce dry skin. Furthermore, another group of prostaglandins known as leukotrienes produced from AA which are a major factor in the promotion of **inflammatory** response and allergies. On the other hand, prostaglandins produced from DGLA such as PGE.sub.1 are anti-**inflammatory** and tend to reduce skin disorders. Moreover, leukotrienes cannot be formed from DGLA, so that their levels will also be modulated by the ratio of DGLA to AA in the skin. Therefore, the ratio of DGLA to AA in the skin will determine which prostaglandins are ultimately produced.

In Table 2 is shown the results of this study with the six groups of individuals using different ratios of activated Omega 6 essential fatty acids and EPA.

TABLE 2

Effect of weight ratios of activated Omega 6 essential fatty acids and EPA on physiological responses related to prostaglandin formation

Group	EPA/GLA Ratio	Energy	Stool	Skin
1	40:1	-1.5 .+-. 0.2		
			-1.3 .+-. 0.2	
				+1.5 .+-. 0.2

2	20:1	+0.8 .+-. 0.3	
		-0.2 .+-. 0.3	
			+1.3 .+-. 0.2
3	10:1	+1.8 .+-. 0.2	
		+1.5 .+-. 0.2	
			+0.5 .+-. 0.2
4	5:1	+1.2 .+-. 0.2	
		+0.7 .+-. 0.3	
			+0.7 .+-. 0.2
5	1:1	-1.2 .+-. 0.3	
		-1.7 .+-. 0.3	
			-0.8 .+-. 0.3
6	0.5:1	-1.7 .+-. 0.2	
		-1.7 .+-. 0.3	
			-1.3 .+-. 0.2

This data shows a number of results. At high ratios of EPA to GLA, energy levels and stool composition (due to diarrhea) worsened for the individuals at the end of the study compared to the start. Both results can be explained by excessive vasodilation caused by too much DGLA formation without a compensating production of AA to maintain an appropriate DGLA to AA balance in the cardiovascular system and gastrointensinal tract. On the other hand, their skin condition improved. This is because the skin is not as senistive to high DGLA levels as are the other systems. Thus at high EPA to GLA weight ratios there are some benefits, although many negative effects are also observed.

The ratio of 10:1 EPA to GLA in this study produced optimal results in the improvement of all three areas (i.e. energy, stool composition and skin).

At the lowest ratios of EPA to GLA, a distinct decrease in the energy, stool composition (due to constipation) and the skin composition (dryness and flaring up of eczema, if an existing condition prior to supplementation) were observed. All of these effects can be explained by the increased levels of AA formation giving rise to a decreased DGLA to AA ratio in these body tissues. Correspondingly, the lowered DGLA to AA ratio would lead to increased levels of vasoconstrictors and pro-**inflammatory** prostaglandins which adversely effect these physiological functions.

These results indicate that there is a specific range of activated Omega 6 essential fatty acids and EPA weight ratios that modulate prostaglandin levels to the benefit of humans, whereas other ratios actually decrease the individual's health. It should be noted that the ratios of activated Omega 6 essential fatty acids and EPA disclosed in the examples of prior art (U.S. Pat. No. 4,681,896) would have caused detrimental effects if given to patients with atopic disorders.

Example 3

6 patients with clinical manifestations of rheumatoid arthritis were place on a daily regimen of 480 mg. EPA and 120 mg. of GLA for three months. At the end of the time period, all clinical signs of rheumatoid arthritis were significantly diminished by joint pain as assessed by their physician and self assessment. This reduction is joint pain can be related to formation of prostaglandins which are anti-**inflammatory**, and the simultaneous suppression of prostaglandins which are pro-**inflammatory**.

Example 4

10 normal subjects were placed on a daily dose of 640 mg. EPA and 80 mg. GLA for 2 weeks. Blood samples were taken, the platelets were isolated. Measurements aggregation of platelets were taken before and after the supplementation program. In these platelet aggregation studies, the isolated platelets were stimulated with collagen (0.5 ug/ml) and a thromboxane A.sub.2 analog (U46619) {500 ng/ml}. The results are shown in Table 3.

TABLE 3

Effects of EPA and activated Omega 6 essential fatty acids on platelet aggregation.

Parameter	Average Before Supplementation	Average after Supplementation	p
Aggregation with U46619	117 .+-. 18	88 .+-. 27	<0.01
Aggregation lag with collagen*	66 .+-. 23	79 .+-. 36	<0.05

n.s. not statistically significant
*lag time in seconds before aggregation

These results can be summarized as follows: There was no change in the platelet count, but there was a statistically significant decrease in platelet aggregation times. This indicates that the decrease in platelet aggregation was due to modulation of prostaglandins in the platelets by the dietary supplementation with EPA and GLA. This modulation of prostaglandins decreased the tendency of these platelets to aggregate when stimulated by an external response.

Example 5

A 0.5 gram soft gelatin capsule containing 15 mg. GLA and 60 mg. EPA. The number of capsules taken on a daily basis to modulate prostaglandins on a short term basis (up to 30 days) would be 4 capsules per day. The amount ingested for a long term basis would be one capsule per day as less activated Omega 6 essential fatty acids are required to maintain the tissue levels of DGLA to AA once they are established.

Example 6

To illustrate the effect of the invention for the treatment of immune disorders, an AIDS patient with clinicial signs of Karposi's sarcoma was put on a dialy regime of 8 0.5 gram soft gelatin capsules containing 15 mg. GLA and 60 mg. of EPA for 60 days. After 60 days, the dosage was increased to 16 capsules per day for the next 6 months. Three months after starting the program, the lesions associated with Karposi's sarcoma had disappeared. The lesions reappeared after eight months from the start of the program. This result shows that a relatively low amount of the invention can cause regression of cancerous lesions such those associated with Karposi's sarcoma.

Example 7

A 0.5 gram soft gelatin capsule containing 8 mg. DGLA and 80 mg. EPA. The number of capsules taken on a daily basis to modulate prostaglandins on a short term basis (up to 30 days) would be 4 capsules per day. The amount taken for a long term basis would be one capsule per day as less activated Omega 6 essential fatty acids are required to maintain tissue levels of DGLA to AA once they are established.

Example 8

Another example of a pharmaceutical composition of the invention is a physiologically compatible intravenous emulsion suitable for injection. 1.0 grams of purified soybean lecithin containing 75% phosphatidylcholine was dispersed in 100 ml. of distilled water buffered with 1 mM phosphate to pH 7.5. To the dispersed lecithin was added 10 grams of oil containing 1.2 grams EPA and 0.3 grams of GLA and 2.25 g of glycerine. The material was emulsified with a Branson W-375 sonifier under a nitrogen atomsphere. The resulting dispersion consisted of an emulsion with an average particle size of 261 nm.

Example 9

To illustrate an example of a food product containing the invention, a dairy emulsion suitable for food use can be made by the dispersion of 1.0 grams of a purified soybean lecithin fraction consisting of 45% phosphatidylcholine in 100 ml. of distilled water. To the dispersed lecithin is added 10 grams of oil containing 1.2 grams of EPA and 0.3 g GLA and 0.01 grams of artificial chocolate flavor. The mixture was homogenized and passed through a microfluidizing apparatus to produce a dispersion with an average particle size of 275 nm.

Example 10

To illustrate the potential of increasing the potency of the invention by fractionation of the active ingredients, in particular the GLA component, the following example is given. 500 g. of refined borage oil was refluxed for 1 hour with a solution of 400 ml. of ethanol, 125 ml. of water, and 115 g. of KOH. The mixture was cooled and 500 ml of crushed ice and 600 ml. of 0.4M H.sub.2 SO.sub.4 was added. The layers were separated, and the upper layer was dried by the addition of 3% by weight of MgSO.sub.4. At this point, the triglycerides of the borage oil have been transformed into free fatty acids. The MgSO.sub.4 was filtered, and the free fatty acids are refluxed for 1 hour with 1000 ml. of methanol and 20 ml. of concentrated H.sub.2 SO.sub.4. After cooling, 1500 ml. of water was added and phases were separated. To the upper phase containing the methyl esters of GLA was added 3% by weight of MgSO.sub.4. The MgSO.sub.4 was filtered and the solution was evaporated to dryness. At this point the concentration of the methyl esters of GLA relative to other fatty acids was 22.1% and the content of linoleic acid was 37.2%, which was similar to that found in the starting borage oil.

To further increase the potency of the methyl ester of GLA the following procedures were used. 1400 g. of urea was dissolved in 5600 ml. of warm methanol/ethanol (2:1 v/v). To the dissolved urea was added 500 g. of the methyl esters of GLA derived from borage oil. The mixture was placed at 0.degree. C. overnight. The mixture was filtered and washed with cold methanol. To eliminate any urea that may have stayed in solution, to the

filtered solution was added 1000 ml. of 0.4M H.sub.2 SO.sub.4 for every 2000 ml. of filtered solution. The upper phase was separated and dried with MgSO.sub.4. The MgSO.sub.4 was filtered, and the solvent were evaporated under vacuum. From the starting 500 g. of unfractionated methyl esters of GLA, the yield was 96 g. Analysis of this fraction by gas liquid chromatography showed that the composition of this fraction was 92.4% GLA and 6.8% linoleic acid. The total recovery of GLA was 80.3%.

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CLM What is claimed is:

1. A method for the reduction of blood pressure levels in a hypertensive mammal, which method comprises administering to the hypertensive mammal a therapeutic, effective amount of a composition which consists essentially of in combination as the active ingredients: a)

eicosapentaenoic acid (EPA) or a salt or ester thereof; and b) gamma **linolenic acid** (GLA) or a salt or ester thereof, the EPA and the GLA employed in a weight ratio of EPA to GLA of from about 2:1 to 8:1.

2. The method of claim 1 wherein said composition includes a carrier for the EPA and the GLA and which carrier is acceptable to the mammal.

3. The method of claim 1 wherein said composition comprises an emulsion.

4. The method of claim 1 wherein said composition is encapsulated in a soft gelatin capsule.

5. The method of claim 1 wherein said EPA compound is selected from the group consisting of: an EPA triglyceride; an EPA monoglyceride; and an EPA methyl ester.

6. The method of claim 1 wherein said GLA compound is selected from the group consisting of: a GLA triglyceride; a GLA monoglyceride; and a GLA methyl ester.

7. The method of claim 1 wherein the amount of the GLA comprises about 80 milligrams.

8. A method for the reduction of blood pressure levels in a hypertensive individual, which method comprises orally administering to the hypertensive individual a therapeutic, effective amount of a composition which consists essentially of as active ingredients: a) eicosapentaenoic acid (EPA); b) gamma **linolenic acid** (GLA); and c) a carrier for the EPA and GLA acceptable to the individual, the EPA and the GLA employed in a weight ratio of EPA to GLA of from about 2:1 to 8:1.

INCL INCLM: 514/549.000
INCLS: 514/552.000; 514/560.000
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TI **Omega-3 fatty acids** in traumatic injury treatment
IN Alexander, J. Wesley, Cincinnati, OH, United States
PA Shriners Hospitals for Crippled Children, United States (U.S. corporation)
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RLI Continuation of Ser. No. US 1989-418690, filed on 2 Oct 1989, now abandoned which is a continuation of Ser. No. US 1989-298825, filed on 18 Jan 1989, now abandoned which is a continuation of Ser. No. US 1987-17326, filed on 20 Feb 1987, now abandoned which is a continuation-in-part of Ser. No. US 1987-2035, filed on 12 Jan 1987, now abandoned
DT Utility

FS Granted

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EXNAM Primary Examiner: Stone, Jacqueline

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ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 2 Drawing Page(s)
AB A composition and method of treating a traumatic injury by improving immunologic response and reducing a hypermetabolic response associated with those suffering from a traumatic injury such as a substantial burn, trauma, major surgery and the like and more particularly to those suffering from a substantial thermal burn injury to the skin or other areas through the administration of the composition of the invention to those suffering from the traumatic injury are disclosed. The composition comprises an intact protein, arginine, carbohydrate, lipid comprising the **omega-3 fatty acids** of fish oil, including eicosapentaenoic acid, and with linoleic acid limited to the amount necessary to prevent an essential fatty acid deficiency thereof and nutritionally necessary vitamins and minerals.

PARN RELATED APPLICATION

This application is a continuation of application Ser. No. 418,690, filed Oct. 2, 1989, which is a continuation of Ser. No. 298,825, filed Jan. 18, 1989, which is a continuation of Ser. No. 17,326, filed Feb. 20, 1987, which is a continuation-in-part of Ser. No. 2,035 filed Jan. 12, 1987, all now abandoned.

SUMM BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a nutritionally fortified pharmaceutical composition for the administration to patients in a hypermetabolic state such as those suffering from a substantial burn, trauma, major surgery and the like, and more particularly to a composition for enteral administration to patients who have encountered a substantial burn injury to the skin or other areas from contact with heat, radiation, electricity or chemicals.

2. Disclosure Statement

Patients suffering from a traumatic injury such as a substantial burn (involving an area greater than about 45 percent of the surface area of a human) become hypermetabolic and are highly susceptible to the development of malnutrition and infection. In 1970 of the patients who survived more than one week, roughly 75 percent died of infectious complications. While substantial improvement has taken place since then, what is needed is an improved nutritionally fortified pharmaceutical composition which will aid the traumatically injured patient manifesting or about to manifest a hypermetabolic state associated with a traumatic injury by attenuating the hypermetabolic state thereby lessening malnutrition and infection associated with a traumatic injury. In patients where the GI tract is still functioning but who are unable to orally take in adequate amounts of nutrients, enteral nutrition is the preferred route of nutritional administration relative the parenteral route.

Generally speaking, enteral nutrition products may be administered orally or by tube feeding routes. The nasogastric, nasoduodenal and nasojejunol routes are nonsurgical. Whereas, the jejunostomy, gastrostomy and esophagostomy are surgically inserted. Enteral nutrition products may also be administered by mouth where the patient is able. The nasoduodenal and nasojejunal are generally used in an unconscious patient and those with an impaired gag reflex in order to minimize the

likelihood of aspiration.

Numerous enteral formulations are utilized in patients with a hypermetabolic state as effected by burns, trauma, major surgery and in those patients with malnutrition syndromes, neoplasms, chronic illnesses and in disorders resulting from prolonged periods of reduced oral intake resulting from cerebral vascular accidents or a comatose state.

ISOCAL is an enteral formulation by Mead Johnson which utilizes casein and soy for its protein source, glucose oligosacchrides for its carbohydrate source and soy oil and medium chain triglycerides (MCT) oil for its lipid source. The composition includes about 19 grams linoleic acid per liter.

OSMOLITE is manufactured by Ross and utilizes as its protein source casein and soy, corn starch for its carbohydrate source and fifty percent MCT oil, forty percent corn oil and ten percent soy oil for its lipid source. The composition includes about 11.5 grams linoleic acid per liter.

ENSURE is manufactured by Ross and utilizes casein and soy for protein source, corn starch and sucrose for a carbohydrate source and corn oil for a lipid source. The composition includes about 19.6 grams linoleic acid per liter.

SUSTACAL manufactured by Mead Johnson utilizes casein and soy for its protein source, corn syrup and sucrose for its carbohydrate source and soy oil for its lipid source. The composition includes about 6.8 grams linoleic acid per liter.

ENSURE PLUS manufactured by Ross is a high protein composition using soy and casein for its protein source, corn starch and glucose for its carbohydrate source and corn oil for its lipid source. The composition includes about 27 grams linoleic acid per liter.

MAGNACAL manufactured by Chesebrough Ponds is a high density composition with 2.0 calories/ml. MAGNACAL utilizes casein for its protein source, corn syrup for its carbohydrate source and soy oil for its lipid source. The composition includes about 59 grams linoleic acid per liter.

TRAUMACAL manufactured by Mead Johnson utilizes casein for its protein source, corn syrup and sucrose for its carbohydrate source and 70 percent soy bean oil and 30 percent MCT oil for its lipid source. The composition includes about 27 grams linoleic acid per liter.

PRECISION ISOTEIN HN is manufactured by Sandoz and utilizes lactalbumin for its protein source, maltodextrin for its carbohydrate source and soy oil and MCT oil for its lipid source. The composition includes about 3.4 grams linoleic acid per liter.

The above enteral compositions fail to provide **omega-3 fatty acids** including eicosapentaenoic acid or a nutritionally fortified pharmaceutical composition containing **omega-3 fatty acids** of fish oil including eicosapentaenoic acid for the reduction or attenuation of hypermetabolic states associated with traumatic injuries such as a burn injury, trauma and major surgery and especially substantial burn injuries.

Therefore, it is an object of this invention to provide a nutritionally fortified pharmaceutical composition and method to aid in the treatment of a traumatic injury with an impaired immune response and an associated hypermetabolic state especially where the hypermetabolic state and immunologic depression are the result of a traumatic injury such as a substantial burn injury.

Another object of this invention is to provide a nutritionally fortified pharmaceutical composition which provides **omega-3 fatty acids** including eicosapentaenoic acid in an amount sufficient to reduce the hypermetabolic resting metabolic state associated with those suffering from a traumatic injury and limits the amount of linoleic acid in the composition to the amount needed for preventing essential fatty acid deficiency.

Another object of this invention is to provide a nutritionally fortified pharmaceutical composition for administration to one suffering from a traumatic injury which decreases the amount of arachidonic acid pathway metabolites formed in a traumatically injured patient thereby enhancing healing of a traumatic injury in the patient in need of such treatment.

Another object of this invention is to provide a method of treating a patient suffering from a traumatic injury, such as a burn injury, by providing to one in need of such treatment **omega-3 fatty acids** including eicosapentaenoic acid in an amount sufficient to attenuate a hypermetabolic response associated with the traumatic injury and limiting the amount of linoleic acid to the amount needed for preventing essential fatty acid deficiency to enhance the healing rate of the burn injury.

Another object of this invention is to provide a nutritionally fortified pharmaceutical composition which provides **omega-3 fatty acids** including eicosapentaenoic acid which enhances the healing rate of a traumatic injury.

Another object of this invention is to provide a nutritionally fortified pharmaceutical composition and method which results in less weight loss, in better skeletal muscle mass maintenance, in a lower resting metabolic expenditure, better opsonic indices, higher splenic weight, lower adrenal weight, higher serum transferrin, lower serum C3 levels and in a better cell mediated immune response for use in a patient with a traumatic injury and its associated hypermetabolic response and immunologic depression.

Another object of this invention to provide a composition which attenuates a hypermetabolic response in a patient in need of such treatment.

The foregoing has outlined some of the more pertinent objects of the present invention. These objects should be construed to be merely illustrative of some of the more pertinent features and applications of the invention. Many other beneficial results can be obtained by applying the disclosed invention is a different manner or modifying the invention within the scope of the disclosure. Accordingly, other objects and a

fuller understanding of the invention may be had by referring to the summary of the invention and the detailed description describing the preferred embodiment in addition to the scope of the invention defined by the claims taken in conjunction with the accompanying formulations and figures.

SUMMARY OF THE INVENTION

The nutritionally fortified pharmaceutical composition and method of treating burns of the present invention are defined by the appended claims with a specific embodiment shown in the included formulations and figures. For purposes of summarizing the invention, the invention relates to a composition and method of treating a traumatic injury by improving immunologic response and reducing a hypermetabolic resting metabolic state associated with those suffering from the traumatic injury such as a substantial burn, trauma, major surgery and the like and more particularly to those suffering from a substantial thermal burn injury to the skin or other areas through the administration of the composition of the invention to those in need of such treatment.

The composition of the invention is a nutritionally fortified pharmaceutical composition for the treatment of a traumatic injury comprising:

an intact protein, in an amount of about 20 to 30 percent of the total energy intake;

carbohydrate, in an amount of about 65-70 percent of the total energy intake; and

fat or lipids, a greater proportion of which do not enter the arachidonic acid pathway, such as oleic acid, yet includes a sufficient amount of linoleic acid to prevent an essential fatty acid deficiency thereof, and **omega-3 fatty acids** of fish oil including eicosapentaenoic acid to provide a total lipid in an amount of about 7-15 percent of the total energy intake, thereby decreasing the amount of arachidonic acid pathway metabolites formed in one suffering from a traumatic injury and reducing the hypermetabolic resting metabolic state and the depressed immune response associated with one suffering from a traumatic injury.

Preferably, the composition includes nutritionally necessary vitamins and minerals.

The intact protein may be derived from the group consisting of lactalbumin, egg albumen or whey, and the like. A property common to the protein which may be used in the invention is that it have a high biologic value. That is, that the protein is better at supporting animal growth relative a protein with a low biologic value. The intact protein supplies about 20 to 30 percent of the total energy intake of the patient. Where the total energy intake of protein is greater than 30 percent, adverse effects, such as weight loss, appear. The optimum range appears to be about 20-25 percent of the total energy intake. The preferred intact protein is whey protein.

The carbohydrate is selected from the group consisting of intact carbohydrates, complex glucose polymers and disaccharides. The intact form of a carbohydrate is derived from cereals, pureed vegetables and complex starches. The intact carbohydrate may be partially hydralized to yield complex glucose polymers. The preferred carbohydrate is a complex

glucose polymers such as POLYCOSE (Ross Laboratories, Columbus, Ohio) or SUMACAL (Chesebrough-Ponds).

The elemental forms of either carbohydrate and/or protein require less digestive capability than the intact macronutrients such as the intact protein or intact carbohydrate. However, the increased number of particles with the elemental formulations will increase osmolality which may potentially increase certain adverse effects such a diarrhea.

Preferably the composition includes arginine in the amount of about 1-3 percent of the total energy intake to enhance healing of a wound or break in the continuity of soft parts of body structure caused by the traumatic injury and to improve immune response. The most preferred amount is 2 percent of the total energy intake.

The amount of intact protein, carbohydrate and lipid is present in the composition within their respective specified range of total energy intake.

The composition may further include the amino acids: cysteine and histidine in an amount sufficient to establish and maintain normal plasma levels. Plasma levels of these amino acids have been found to be subnormal in patients suffering from a substantial burn injury.

The composition of the invention preferably includes nutritionally necessary expedients such as vitamins and minerals. Commercial preparations such as NUTRISOURCE Vitamins and NUTRISOURCE minerals by Sandoz are readily available.

The lipid provides 7-15 percent of the total calories and always includes a limited amount of linoleic acid sufficient to prevent an essential fatty acid deficiency thereof. The lipid also always includes **omega-3 fatty acids** of fish oil including eicosapentaenoic acid in an amount sufficient to attenuate or reduce a hypermetabolic resting metabolic state resulting from a traumatic injury, especially a substantial burn injury. Any remaining lipid which is needed to attain the 7-15 percent of the total calories is provided by lipids which do not enter the arachidonic acid pathway.

The remaining lipid of the inventive composition may be provided by long-chain fatty acids which are present in butterfat and vegetable oils. The vegetable oils include peanut oil, olive oil, safflower oil and sunflower oil and the like. The amount of linoleic acid in vegetable oils must be considered when determining the amount to be used in the composition without providing any excess over that required to prevent a deficiency thereof. The lipid may be further provided by medium chain triglycerides (MCT). When medium chain triglycerides are used an effective amount of essential fatty acids must be either included in the composition of the invention or otherwise provided to the patient in order to prevent essential fatty acid deficiency. Preferably, the **omega-3 fatty acids** including eicosapentaenoic acid are provided by fish oil. **Omega-3 fatty acids** C20:5 eicosapentaenoic acid and C22:6 docosahexaenoic acid are typical of fish oil. More specifically, the **omega-3 fatty acids** of fish oil include eicosapentaenoic acid, docosahexaenoic aid, docosapentaenoic acid and **linolenic acid**. The exact percentages of fatty acids in fish oil may vary between species.

The fatty acid components of fish oil include:

C14:0 (Myristic) 7.01%; C14:1--0.36%;

C15:0--0.63%; C15:1 and C15:0 (BR)--0.14%;

C16:0--17.03%; C16:1 (palmitoleic)--8.68%;

C17:0--2.24%; C17:1 and C17:0 (BR)--1.11%;

C18:0 (Stearic)--4.79%; C18:1 (oleic)--12.75%;

C18:2 (linoleic)--omega 6--1.79%; 18:3 (linolenic)--omega 3--0.46%;

C20:0 (arachidic)--2.19%;

C20:1--1.57%; C20:5 (eicosapentaenoic)--omega-3--17.43%;

C22:1--0.73%;

C22:5 (docosapentaenoic)--omega-3--3.20%;

C22:6 (docosahexaenoic)--omega-3--16.84%.

See Sanders, T. A. B. and Roshanai, F., Clinical Science, Vol. 64 pp. 91-99, 1983 the disclosure of which is incorporated herein as if fully set forth herein for a discussion of fish oil. Cod liver oil also contains **omega-3 fatty acids**.
However, because of the amount necessary to provide sufficient **omega-3 fatty acids**, toxicity from high levels of Vitamin A may arise. Therefore, the use of cod liver oil as a source of **omega-3 fatty acids** is less desirable. Further, any arachidonic acid present in any oil would may have to be considered relative the amount of linoleic acid since arachidonic acid is derived from linoleic acid and enters the arachidonic acid pathway. A mechanical mixture of the **omega-3 fatty acids** as present in fish oil would be expected to perform substantially as fish oil which naturally includes **omega-3 fatty acids**.

In a preferred embodiment, the lipid consists of a mixture of substantially equal amounts of nonprotein calories provided by fish oil which contains eicosapentaenoic acid and by safflower oil, where the lipid supplies about 15 percent of the nonprotein calories.

Safflower oil, commercially available as MICROLIPID (Organon Inc., West Orange, N.J.) contains about 74 percent linoleic acid. Fish oil and marine oils contain high levels of eicosapentaenoic acid. MaxEPA (R.P. Scherer Corp., Clearwater, Fl.) is a commercially available fish oil which contains about 18 percent eicosapentaenoic acid.

A more specific embodiment of the invention embraces a nutritionally fortified pharmaceutical enteral composition for the treatment of a traumatic injury comprising:

whey protein, in an amount of about 20-25 percent of the total energy intake;

arginine, in an amount of about 2 percent of the total energy intake to enhance wound healing;

complex glucose polymers, in an amount of about 65 percent of the total energy intake;

a lipid in an amount of about 12 percent of the total energy intake, comprising a mixture of substantially equal amounts of nonprotein calories provided by vegetable oil with a sufficient amount of linoleic acid to prevent an essential fatty acid deficiency thereof and fish oil

in an amount sufficient to reduce the hypermetabolic resting metabolic state associated with one suffering from a traumatic injury; and

nutritionally necessary vitamins and minerals.

Preferably, the composition further includes cysteine and histidine which each supply about one-half percent (0.5 percent) of the total energy intake of the patient to provide normal plasma levels.

Preferably the mixture of substantially equal amounts of nonprotein calories provided by safflower oil and fish oil, in an amount of about 12 percent of the total energy intake of the patient.

Preferably the arginine is supplied in the amount of about 1-3 of the total energy intake to enhance healing of a wound or break in the continuity of soft parts of body structure caused by the traumatic injury and improve immune response.

Enteral support is the preferred route of nutrient delivery. However, diarrhea often accompanies this route, especially continuous nasogastric hyperalimentation. Diarrhea disturbs fluid and electrolyte balance and worsens nutritional status. Tube feeding hyperosmolality and serum albumin levels are the suggested key causative factors in tube feeding-induced diarrhea via hypertonicity mechanisms.

In another embodiment of the invention, the low fat composition provides for the treatment of a traumatic injury by decreasing the risk of diarrhea when combined with Vitamin A and is enterally administered preferably within 48 hours after the inflection of the traumatic injury and most preferably within 24 hours. The enteral composition comprises:

an intact protein, in an amount of about 20 to 30 percent of the total energy intake;

carbohydrate, in an amount of about 65 to 70 percent of the total energy intake;

Vitamin A in an amount sufficient to substantially decrease the risk of diarrhea;

lipids, in an amount of about 7 to 15 percent of the total energy intake comprising an amount of linoleic acid sufficient to prevent an essential fatty acid deficiency thereof and eicosapentaenoic acid in an amount sufficient to reduce a hypermetabolic resting metabolic state associated with one suffering from a traumatic injury, especially a substantial burn injury.

The enteral composition which substantially decreases the risk of diarrhea usually associated with tube feeding formulas, includes an amount of Vitamin A sufficient to provide 5,000 I.U. per day for enteral administration to a child from 9 months through 3 years of age. For a patient older than 3 years, including an adult, the enteral composition includes an amount of Vitamin A greater than about 10,000 I.U. per day. Possible toxic effects of large doses of Vitamin A exist, however amounts up to 50,000 I.U. daily are considered safe for up to 8 months. Preferably the enteral composition utilizes whey for the intact protein, a mixture of substantially equal amounts of nonprotein calories provided

by fish oil and by safflower oil, where the lipid supplies about 15 percent of the nonprotein calories for the lipid and includes arginine, supplying about 2 percent of the total energy intake, and cysteine and histidine each supplying about one-half percent of the total energy intake.

The invention may also be incorporated into a method of treating a traumatic injury, especially where the traumatic injury is a substantial thermal burn injury to the skin and involved areas. The traumatic injury is treated by administering an amount of a composition comprising **omega-3 fatty acids** of fish oil including eicosapentaenoic acid sufficient to reduce or inhibit a hypermetabolic resting metabolic state associated with those suffering from a traumatic injury such as a substantial burn, trauma, major surgery and the like and especially to those suffering from a substantial thermal burn injury to the skin or other areas.

Preferably, the method includes administering to one in need of such treatment **omega-3 fatty acids** of fish oil including eicosapentaenoic acid in an amount sufficient to inhibit the onset of a hypermetabolic response associated with traumatic injury and improve immune response as soon as possible after the injury to maximize the benefit of inhibiting or attenuating the hypermetabolic response and to lessen the risk of infection by improving immunologic response. Increases in resting energy expenditure and catabolic hormones are reduced when enteral feeding is implemented immediately after a burn. Conversely, when enteral restriction is imposed while passively waiting for post-traumatic ileus to resolve, a noticeable hypermetabolic syndrome follows. Typically, metabolic rate is normal on the first two days following burn injury and then climbs dramatically over the course of several weeks. However, when burns exceed about 50 percent total body surface area or in some cases of sepsis, metabolic rate plateaus or may even decrease. Evidence appears to support a chronic elevation of catecholamines which act as the dominant stimulus of the hypermetabolic response to burns. Increased plasma catecholamines and high urinary catecholamine excretion have been correlated with burn size and metabolic rate. Enteral feeding should begin within 48 hours postburn and preferably within 24 hours postburn and most preferably within 6 hours postburn. The suppression of hypermetabolic response is evidenced by decreased resting energy expenditure, positive nitrogen balance and normal serum concentration of the catabolic hormones. Other benefits from early GI feeding include the ability to immediately satisfy nutritional requirements along with promoting bowel mucosal integrity and improved tube feeding tolerance. Preferably, the enteral composition is administered by utilizing a nasogastric tube connected to low Gomco suction while simultaneously enterally feeding via a nasoduodenal tube within 24 hours postburn.

Preferably the composition includes the **omega-3 fatty acids** of fish oil eicosapentaenoic and docosahexaenoic acids. Most preferably the composition includes fish oil containing the **omega-3 fatty acids** eicosapentaenoic acid and docosahexaenoic acid.

More specifically, the method of treating burn injuries further includes restricting the intake of linoleic acid such that only the amount necessary to meet the essential fatty acid requirement is met thereby

minimizing the amount of arachidonic acid pathway metabolites associated with linoleic acid. The amount of linoleic acid necessary to prevent a deficiency is about 3-4 percent of the total energy intake.

Preferably, the method of treating a traumatic injury by enterally administering to one in need of such treatment a composition of the invention which comprises:

an intact protein, in an amount of about 20 to 30 percent of the total energy intake;

arginine, in an amount sufficient to enhance wound healing and improve immune response;

carbohydrate, in an amount of about 65-70 percent of the total energy intake; and

fat or lipids, comprising a greater proportion of lipids which do not enter the arachidonic acid pathway, such as oleic acid, yet including a sufficient amount of linoleic acid to prevent an essential fatty acid deficiency thereof, and **omega-3 fatty acids** of fish oil including eicosapentaenoic acid where the total lipid is supplied in an amount of about 7-15 percent of the total energy intake, thereby decreasing the amount of arachidonic acid pathway metabolites formed in one traumatically injured and reducing the hypermetabolic resting metabolic state associated with one suffering from a traumatic injury. Preferably, the composition may include nutritionally necessary vitamins and minerals.

A more specific embodiment of the method of treating a traumatic injury by enterally administering to one in need of such treatment a composition of the invention which comprises:

whey protein, in an amount of about 20 to 25 percent of the total energy intake;

arginine, in an amount sufficient to enhance wound healing and improve immune response;

complex glucose polymers, in an amount of about 65-70 percent of the total energy intake; and

a lipid, comprising a mixture of substantially equal amounts of nonprotein calories provided by vegetable oil having a sufficient amount of linoleic acid to prevent an essential fatty acid deficiency thereof and fish oil which contains eicosapentaenoic acid in an amount sufficient to decrease the amount of arachidonic acid pathway metabolites formed in one suffering from a traumatic injury and to reduce the hypermetabolic resting metabolic state associated with one suffering from a traumatic injury.

Preferably, the method includes administering the composition of the invention containing nutritionally necessary vitamins and minerals.

Preferably, the method includes enterally administering the composition further containing cysteine and histidine which each supply about one-half percent of the total energy intake of the patient.

Preferably, the method includes enterally administering the composition where the mixture of substantially equal amounts of nonprotein calories provided by safflower oil and fish oil is supplied in an amount of about

7-15 percent of the total energy intake of the patient.

Preferably, the method includes enterally administering the composition further containing arginine in an amount of 1-3 percent of the total energy intake to enhance healing of a wound or break in the continuity of soft parts of body structure caused by the traumatic injury and improve immune response.

The preferred method of administering the composition of the invention includes the continuous enteral administration to one in need of such treatment. That is, the continuous enteral administration is preferred over the intermittent enteral administration of the composition of the invention. The most preferred method includes enterally administrating the composition of the invention to one in need as soon as possible after the infliction of the traumatic injury.

The composition of the invention is intended to be the sole source of dietary energy or total energy intake (protein, carbohydrate and lipid) for the time the patient is unable to take food by mouth. Typically, a patient who has suffered traumatic injury, especially a traumatic burn injury, is not able take food by mouth for a period of time following the injury.

The foregoing has outlined rather broadly the more pertinent and important features of the present invention in order that the detailed description of the invention that follows may be better understood so that the present contribution to the art can be more fully appreciated. Additional features of the invention will be described hereinafter which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and the specific embodiment disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims.

DRWD BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the nature and objects of the invention, reference should be had to the following detailed description taken in connection with the accompanying drawings in which:

FIG. 1 is a comparison of the metabolic rates of different groups of animals receiving similar diets, except for the amino acid composition, by continuous administration;

FIG. 2 is a comparison of resting metabolic rates of burned animals receiving different types of lipids in their diets;

FIG. 3 is a simplified display of the metabolites of arachidonic acid; and

FIG. 4 is a comparison of the dienoic and trienoic prostaglandin pathways.

Similar reference characters refer to similar parts throughout the several views of the drawings.

DETD DETAILED DISCUSSION

The present invention discloses a nutritionally fortified pharmaceutical

composition and method which is suitable for using in patients suffering from traumatic injuries, such as burn injuries which initiate a hypermetabolic state.

FIG. 1 is a comparison of the resting metabolic expenditure (RME) of different groups of animals continuously receiving similar diets (isocaloric, isontrogenous and isovolemic) except for the amino acid composition. NOVAMINE, a commercially available amino acid composition was administered intragastrically (IG) and intravenously (IV). An Amino Acid group was administered intragastrically (IG) and comprised crystalline amino acids in the same amounts found in whey protein. As illustrated at FIG. 1, all groups demonstrated an increase in RME, however, the group receiving whey protein clearly demonstrated whey protein's ability to keep the resting metabolism nearest to the preburn RME. The crystalline amino acids present in the same amounts as found in whey protein also restricted the metabolic rate increase, but not to the extent of the intact whey protein.

FIG. 2 is a comparison of resting metabolic rates of burned animals receiving similar diets but different types of lipids: MICROLIPID (74 percent linoleic acid and 15 percent oleic acid), linoleic acid, oleic acid and MaxEPA. The lipid comprised 10 percent of the total energy intake. As clearly indicated the hypermetabolic response was inhibited for the group of animals which received MaxEPA (fish oil). It is theorized that linoleic acid and other **omega-6 fatty acids** enter the arachidonic acid pathway thereby increasing the production of certain metabolites (dienoic prostaglandins) which appear to have adverse effects on the healing process of one suffering from a traumatic injury, such as a substantial burn. Among the metabolites are the dienoic prostaglandins PGE.sub.2, PGI.sub.2 and TXA.sub.2. FIG. 2 illustrates that arachidonic acid pathway metabolites contribute to the adverse effects of lipids following burn injury, as evidenced by the differences in inhibition of the hypermetabolic state by the compared lipids.

FIG. 3 is a simplified display of the metabolites of arachidonic acid. HPETE indicates 5(S)-hydroperoxy-6,8,14-eicosatetraenoic; LT=leukotriene; PG=prostaglandin; MDA=malondialdehyde; HHt=(12S)-12-hydroxy-5,8,10-hepatodecatrienoic and TX=thromboxane. It appears that the balance between TXA.sub.2 and PGI.sub.2 takes part in the regulation of vascular responses, platelet aggregation and thrombosis. PGE.sub.2 by itself is a vasodialtory prostaglandin that may cause edema formation. However, PGE.sub.2 in combination with bradykinin, histamine, and/or degradation products of a complement pathway present a synergistic potentiation in the formation of edema. More importantly, PGE.sub.2 is a potent depressant of immune response and has feedback mechanisms that inhibit the activities of interleukin-1 and interleukin-2 as well as stimulating the development and activity of suppressor T cells. Accordingly, the biological effects manifested by the metabolites of the arachidonic acid pathway explains the adverse effects manifested by their presence.

FIG. 4 is a comparison of the dienoic and trienoic prostaglandin pathways. TX=thromboxane. The trienoid prostaglandin metabolites PGI.sub.3, PGE.sub.3 and TXA.sub.3 are equivalent to the major metabolites PGE.sub.2, PGI.sub.2 and TXA.sub.2 of the dienoid prostaglandins. However, PGE.sub.3 and TXA.sub.3 lack potency when compared with their dienoid counterparts and block the action of PGE.sub.2 and TXA.sub.2. Thus, regulation of dietary lipids by controlling the intake of **omega-6 fatty**

acids controls the synthesis of dienoic prostaglandins and therefor the adverse effects associated therewith.

A more through discussion is set forth in: Alexander, J. Wesley, Nutrition and Infection, Arch. Surg., Volume 21, pp. 966-972 (August 1986); Alexander, J. Wesley, The Importance of Lipid Type in the Diet after Burn Injury, Ann. Surg., Volume 204, No. 1, pp. 1-8 (July 1986) and, Gottschlich, Michele and Alexander, J. Wesley, Fat Kinetics and Recommended Dietary Intake in Burns, J. Parenteral and Enteral Nutrition, Volume 11, No. 1, pp. 80-85 (1987), and Trocki O., Saito H., Gonce S. J., Heyd T. J., Alexander J. W., Effects of Dietary Lipids on Postburn Metabolic and Immune Response, JPEN 10:50, 1986 each of which is incorporated herein by reference as if fully set forth herein.

Fish oil which is rich in trienoic prostaglandin precursors exerts beneficial effects on gastrointestinal, immunologic and **inflammatory** response when compared to fat from vegetable oils which are largely composed of dienoic prostaglandin precursors in the management of burns.

In one embodiment of the invention an enteral composition utilized as its protein source, 87 percent whey protein with 9 percent arginine, 2 percent cysteine and 2 percent histidine (100 percent of the protein) at 57 grams per liter. The protein source supplied about 20 percent of the total energy intake required by the patient. The carbohydrate source was maltodextrin which supplied about 85 percent of the nonprotein calories (about 68 percent of the total energy intake) and was added at a rate of 164 grams per liter. The lipid source consisted of fish oil and safflower oil providing 15 percent of the nonprotein calories (about 12 percent of the total calories) with substantially equal amounts of nonprotein calories provided by the fish oil (MaxEPA), which contained eicosapentaenoic acid and docosahexaenoic acid, and by the safflower oil. The fish oil-safflower mixture was added at a rate of 13 grams per liter. The fish oil-safflower oil mixture included 4.8 grams linoleic acid per liter. The composition of the invention supplies about 1000 kcalories per liter and had an osmolality of 549 mOsm/Kg water.

An enteral composition of the invention was prepared as set forth below:

Modular Tube Feeding-Shriners Burns Institute

Ingredients	Amount		CHO	PRO	FAT	KCAL
Sterile Water	750	ml				
MaxEPA (fish oil)	6	ml	0	0	6	54
MICROLIPID	9	ml	0	0	4.4	39
PROMIX	62	g	3.3	50	2.6	236
SUMACAL	149	g	142	0	0	568
Arginine HCL	5	g	0	5	0	20
Histidine	1	g	0	1	0	4
Cysteine	1	g	0	1	0	4
NUTRISOURCE Minerals	24	g	6	0	0	24
NUTRISOURCE Vitamins	20	g	18	0	0	72
Vitamin A (50,000 units/ml)	0.1	ml	0	0	0	0
TOTAL			163.3	57	13	1021

The sterile water was measured and poured into a blender such as a Waring blender. The MaxEPA (R. P. Scherer Corporation) (fish oil) was measured using a pipette and/or graduated cylinder. The MICROLIPID (Chesebrough-Ponds) preparation was vigorously shaken and measured utilizing a pipette. Both of these liquids are added to the blender. PROMIX (Navaco) was weighed out and added to the blender. SUMACAL (Chesebrough-Ponds) was weighed and add to liquids in blender. The Arginine HCL, Histidine and Cysteine were individually weighed and added to the liquids in the blender. Twenty four grams of NUTRISOURCE (Sandoz) Minerals and 20 grams of NUTRISOURCE (Sandoz) Vitamins were added to the liquids in the blender. Vitamin A was measured and added to the liquids in the blender. All ingredients were mixed in the blender at low speed for about 30 seconds taking caution not to over mix. The foam was allowed to settle. To check proper recipe level, the liquid was poured into a 2000 ml flask and more sterile water was added if necessary to bring up to the correct 1000 ml level. The formula provided about 1000 Kcal. The formula was transferred into brown bottle and refrigerated. After 24 hours any remaining formula is discarded. To prepare an enteral composition of the invention which decreases the risk of diarrhea, an amount of Vitamin A is added to the composition as described above.

CALCULATION FORMULA FOR 1000 ml

Kcal needs = 1000
Protein needs (20% of Kcal)
= 200 Kcal or 50 g protein
Arginine needs (2% of Kcal)
= 20 Kcal or 5 g arginine
Histidine needs (0.5% of Kcal)
= 5 Kcal or 1 g
Cysteine needs (0.5% of Kcal)
= 5 Kcal or 1 g
1000 Kcal
-230 Kcal
(arginine + histidine + cysteine + Promix)
770 Kcal
from nonprotein sources
Total fat
= 15% of nonprotein calories
= .15 .times. 770 = 115 Kcal or 13 g fat
Fat from Maxepa
= 7% of nonprotein calories
= .07 .times. 770 = 54 Kcal or 6 g Maxepa
= 6 ml
Fat from Microlipid
= 8% of nonprotein calories
= 13 - (2.6 + 6)
= 4.4 g of Microlipid
= 9 ml
Carbohydrate
= 85% of nonprotein calories
= .85 .times. 770 = 655 Kcal or 163 g carbohydrates
230 Kcal (protein) + 115 Kcal (fat) + 655 Kcal
= 1000 Kcal
(carbohydrate)

The composition may further include about 220 mg/day zinc sulfate where the occurrence of a suboptimal serum level is encountered in a traumatically injured patient. The composition may further include about

1 gm of vitamin C per day.

Diarrhea is a frequent problem in the use of enterally administered enteral compositions. In an effort to determine the cause, a study was conducted. Diarrhea was defined as greater than 4 liquid bowel movements per day or a large (greater than 200 g) liquid stool. Of the 20 patients studied, 6 (30%) developed diarrhea. Stool cultures were negative for pathogenic organisms. The mean total body surface area burn of those who developed diarrhea was 55 percent (range 12-89%) whereas those without diarrhea had a mean burn size of 43 percent (range 28-71%) (not significant). Results demonstrated a significant relationship between dietary lipid content and diarrhea. Implementation of tube feeding within 48 hours postburn was associated with a decreased incidence of diarrhea. The risk of diarrhea was most closely associated with inadequate Vitamin A intake. Surprisingly, tube feeding osmolality, systemic antibiotics, or hypoalbuminemia did not have an adverse effect on intestinal absorption. Various enteral feeding products were compared. This study demonstrated a highly significant effect of the lowfat enteral composition of the invention augmented with vitamin A, as set forth above, in decreasing the risk/incidence of diarrhea associated with enteral feeding.

Numerous methods and formulas exist for determining the daily caloric needs of traumatically injured patients. Formulas such as the Curreri formula has been found workable in initially projecting energy needs in burn patients: (25 kcal.times.kg body weight)+(40 kcal.times.percent burn). While helpful in estimating peak energy needs, the Curreri formula leads to overfeeding as the patient progresses. The failure of the Curreri formula and other conventional formulas, is that they do not account for many effectors of energy balance over time. The object of any formula must be to provide adequate calories for positive nitrogen balance and satisfactory maintenance of body weight without overfeeding.

To attain this object, energy expenditure is ascertained by measuring oxygen consumption of the patient by means known in the art. However, other energy requiring activities such as physical therapy, hydrotherapy and dressing changes can elevate energy needs and must be considered. Therefore, providing nutritional intake equivalent to 1.3 times the measured energy expenditure at rest is associated with conditions of positive nitrogen balance and satisfactory maintenance of body weight in the majority of burn patients.

The composition may be prepared in a dry form, with the lipid in an accompanying vial, for subsequent reconstitution by the addition of water and lipid. Such methods are well known in the art. Also, the composition may be prepared as needed or prepared as a ready-to-use composition by methods well known in the art.

The present disclosure includes that contained in the appended claims as well a that of the foregoing description. Although this invention has been described in its preferred form with a certain degree of particularity, it is understood that the present disclosure of the preferred form has been made only by way of example and that numerous changes in the details of construction and the combination and arrangement of parts may be resorted to without departing from the spirit and scope of the invention.

CLM What is claimed is:

1. A method of treating a traumatic injury which manifests a resting hypermetabolic state by administrating to one suffering from such an injury, a composition comprising an amount of **omega-3 fatty acids** of fish oil sufficient to reduce the resting hypermetabolic state associated with the traumatic injury.

2. A method of treating a traumatic injury which manifests a resting hypermetabolic state by administrating to one suffering from such an injury, a composition comprising an amount of **omega-3 fatty acids** of fish oil sufficient to reduce the resting hypermetabolic state associated with the traumatic injury; and an amount of linoleic acid limited to only the amount necessary to meet the essential fatty acid requirement of linoleic acid thereby reducing the amount of arachidonic acid pathway metabolites associated with the metabolism of linoleic acid.

3. A method of treating a traumatic injury by reducing a hypermetabolic resting metabolic state associated with the traumatic injury by enterally administering to one in need of such treatment an effective amount of a composition comprising: an intact protein, in an amount of about 20 to 30 percent of the total energy intake; carbohydrate, in an amount of about 65 to 70 percent of the total energy intake; lipids, in an amount of about 7 to 15 percent of the total energy intake, comprising an amount of linoleic acid sufficient only to prevent an essential fatty acid deficiency thereof thereby reducing the amount of arachidonic acid pathway metabolites in the traumatically injured patient, and **omega-3 fatty acids** of fish oil including eicosapentaenoic acid in amount sufficient to reduce a hypermetabolic resting metabolic state associated with one suffering from a traumatic injury.

4. The method of claim 1 wherein the method further includes continuous enteral administration of the composition to one in need of such treatment.

5. The method of claim 3 wherein the traumatic injury is a substantial thermal burn injury to the skin.

6. The method of claim 4 further administering the composition as soon as possible within 24 hours after the infliction of the traumatic injury.

7. The method of claim 3 wherein the composition includes arginine in an amount sufficient to enhance healing of a wound or break in the continuity of soft parts of body structure caused by the traumatic injury.

8. The method of claim 3 wherein the **omega-3 fatty acids** are provided by fish oil.

9. A method of treating a traumatic injury by reducing a hypermetabolic resting metabolic state associated with the traumatic injury by enterally administering to one suffering from a traumatic injury an effective amount of a composition comprising: whey protein, in an amount of about 20 to 25 percent of the total energy intake; arginine in an amount sufficient to enhance healing of a wound or break in the continuity of soft parts of body structure caused by the traumatic injury; carbohydrate, in an amount of about 65 to 70 percent of the total energy intake where the carbohydrate is complex glucose polymers; lipids, where the lipid supplies about 12 percent of the total energy intake and is provided by a mixture of substantially equal amounts of nonprotein calories provided by fish oil which comprises **omega -3 fatty acids** and by vegetable oil with a

sufficient amount of linoleic acid to prevent an essential fatty acid deficiency thereof, thereby reducing the amount of arachidonic acid pathway metabolites formed in the traumatically injured patient and reducing a hypermetabolic resting metabolic state associated with one suffering from a traumatic injury.

10. The method of claim 9 wherein the traumatic injury is a substantial thermal burn injury to the skin.

INCL INCLM: 514/002.000
INCLS: 514/021.000; 514/023.000; 514/054.000; 514/396.000; 514/560.000; 514/561.000; 514/562.000; 514/867.000; 424/523.000; 424/DIG.013
NCL NCLM: 514/002.000
NCLS: 424/523.000; 424/DIG.013; 514/021.000; 514/023.000; 514/054.000; 514/396.000; 514/560.000; 514/561.000; 514/562.000; 514/867.000
IC [5]
ICM: A61K037-02
ICS: A61K031-70; A61K035-60; A61K031-20
EXF 514/2; 514/21; 514/23; 514/54; 514/396; 514/560; 514/561; 514/562; 514/867; 424/523; 424/DIG.13; 530/833
ARTU 184
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 31 OF 33 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 5
AN 1991:250792 BIOSIS
DN BA91:131347
TI INFLUENCE OF AN **OMEGA-3 FATTY ACID** -ENRICHED RATION ON IN-VIVO RESPONSES OF HORSES TO ENDOTOXIN.
AU HENRY M M; MOORE J N; FISCHER J K
CS DEP. LARGE ANIMAL MED., COLLEGE VETERINARY MED., UNIV. GEORGIA, ATHENS, GA. 30602.
SO AM J VET RES, (1991) 52 (4), 523-527.
CODEN: AJVRAH. ISSN: 0002-9645.
FS BA; OLD
LA English
AB Because certain **inflammatory** processes are dependent on the fatty acid composition of the cellular membrane, dietary manipulations that replace **.omega.-6 fatty acids** with **.omega.-3 fatty acids** may modify **inflammatory** responses. We investigated the effect of supplemental dietary linseed oil, containing the **.omega.-3-fatty acid**, .alpha.-**linolenic acid**, on in vivo responses of horses to endotoxin. One group of horses (n = 6) was fed a control pelleted ration (0% linseed oil), and another group of horses (n = 6) was fed an 8% linseed oil pelleted ration. After 8 weeks of consuming these rations, all horses were given 0.03 .mu.g of Escherichia coli 055.B5 endotoxin/kg of body weight, infused over 30 minutes. Horses were monitored over 24 hours. Compared with baseline values within each ration group, endotoxin infusion caused significant ($P < 0.05$) increase in rectal temperature, heart rate, and plasma concentration of thromoboxane B2, 6-keto-prostaglandin F1.alpha., and fibrinogen and significant ($P < 0.05$) decrease in total WBC count. Compared with baseline values within each ration group, endotoxin infusion failed to cause significant changes in prothrombin, activated partial thromboplastin, thrombin, or whole blood recalcification times, serum concentration of fibrin degradation products, PCV, or plasma total protein concentration. Before and after endotoxin infusion, horses given the linseed oil ration had longer mean whole blood recalcification time and activated partial thromboplastin time than did horses fed the control ration.
CC Biochemical Studies - Lipids 10066
Biochemical Studies - Carbohydrates 10068
Nutrition - Prophylactic and Therapeutic Diets *13218

Nutrition - Lipids *13222
Toxicology - General; Methods and Experimental *22501
Physiology and Biochemistry of Bacteria 31000
Medical and Clinical Microbiology - Bacteriology *36002
Veterinary Science - Pathology *38004
Veterinary Science - Microbiology *38006
BC Enterobacteriaceae 04810
Equidae 86145
IT Miscellaneous Descriptors
ESCHERICHIA-COLI LINSEED OIL

L13 ANSWER 32 OF 33 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 6
AN 89116803 EMBASE
DN 1989116803
TI Summary of the NATO Advanced Research Workshop on Dietary .omega.3 and .omega.6 **Fatty Acids**: Biological Effects and Nutritional Essentiality.
AU Simopoulous A.P.
CS Division of Nutritional Sciences, International Life Sciences Institute Research Foundation, Washington, DC 20036, United States
SO Journal of Nutrition, (1989) 119/4 (521-528).
ISSN: 0022-3166 CODEN: JONUAI
CY United States
DT Journal
FS 007 Pediatrics and Pediatric Surgery
017 Public Health, Social Medicine and Epidemiology
021 Developmental Biology and Teratology
029 Clinical Biochemistry
LA English
SL English
AB A number of human studies presented at the workshop indicate that the premature infant at birth is biochemically deficient in docosahexaenoic acid (DHA) in both the brain and liver phospholipids, and that DHA is essential for normal visual acuity. The amount of DHA necessary to maintain normal amounts of the liver and brain phospholipids postnatally is 11 mg/kg daily. Elderly patients on long-term gastric tube feedings and others on long-term intravenous fluids and on total parental nutrition are particularly prone to deficiencies of .alpha.-**linolenic acid**, eicosapentaenoic acid (EPA) and DHA. The amount estimated to prevent deficiencies in the elderly are 800-1100 mg/d of .alpha.-**linolenic acid** and 300-400 mg/d of EPA and DHA combined. Preliminary data indicate that children with malnutrition and mucoviscidosis, women with toxemia, and elderly people have decreased amounts of DHA in plasma phospholipids. The .**omega.3 fatty acids** lower triglycerides and, at high levels, lower cholesterol. The anti-aggregatory, anti-thromboctic and anti-**inflammatory** properties of .**omega.3 fatty acids** have been confirmed, and a dose-response curve is emerging. Despite the increase in bleeding time, no clinical evidence of bleeding has been noted by the investigators in any of the studies. Clinical trials are necessary in order to precisely define the dose and mechanisms involved in defining the essentiality of .**omega.3 fatty acids** in growth and development and their beneficial effects in coronary heart disease, hypertension, **inflammation**, arthritis, psoriasis, other autoimmune disorders, and cancer.
CT Medical Descriptors:
*metabolism
brain
growth
liver
newborn
review
human

priority journal
Drug Descriptors:
*docosahexaenoic acid
*unsaturated fatty acid
RN (docosahexaenoic acid) 25167-62-8, 32839-18-2

L13 ANSWER 33 OF 33 USPATFULL
AN 87:48882 USPATFULL
TI Rapid acting intravenous emulsions of **omega-3 fatty acid** esters
IN Ward, Michael V., McHenry, IL, United States
Cotter, Richard, Libertyville, IL, United States
PA Baxter Travenol Laboratories, Inc., Deerfield, IL, United States (U.S. corporation)
PI US 4678808 19870707 <--
AI US 1985-787741 19851015 (6)
DT Utility
FS Granted
REP US 4513008 Apr 1985 514/560.000 Revici et al.
REN Needleman, P. et al., "Triene Prostaglandins" Proc. Natl. Acad. Sci. 76(2): 944-948, 1978.
Bang, H. et al., "The Composition of Food Consumed by Greenland Eskimos"
Acta Med Scand. 200:69-73, 1976.
Sanders, T. et al., "A Comparison of the Influence on Plasma Lipids on &
Platelet . . . " Bnt. J. Nut. 50:521,522, 1983.
Goodnight, S. et al., "The Effects of Dietary .omega.3 Fatty Acids . . .
" Blood, 58(5): 880-885, 1981.
Thorngren, M. et al., "Effects of 11-Week Increase in Dietary Eicosapentaenoic Acid" The Lancet Nov. 1981, 1190.
Lorenz, R. et al., "Platelet Function . . . " Circulation 67(3):504-511,
1983.
EXNAM Primary Examiner: Brown, Johnnie R.; Assistant Examiner: Rollins, John W.
LREP Fentress, Susan B., Flattery, Paul C.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
AB Described are lipid emulsions of marine oils comprising high concentrations of **omega-3-fatty acid** esters and low concentrations of free fatty acids for intraveneous administration for the treatment of thrombic disease states. More specifically, a lipid emulsion for parenternal use is provided comprising an emulsifier, water and a marine oil comprising an omega-3-fatty ester in which the concentration of the free fatty acid in
the emulsion is below about 5 meq/l.

SUMM BACKGROUND OF INVENTION

This invention relates to a therapeutic composition, methods for its preparation and for its use. More particularly, this invention relates to an emulsion of marine oil for treatment of thrombotic disease.

The therapeutic use of intravenous (IV) lipid emulsions in the clinically ill has its origin in antiquity. Physicians originally attempted infusions of olive oil and milk into the blood stream of critically ill patients in the 1600s and 1700s. The therapeutic reason for these infusions was to prevent starvation, often the deciding factor
in the survival of such patients. Lipid is an attractive nutritional high calorie source (9kcal/g) as compared to carbohydrate (4kcal/g). These early experiments were unsuccessful due to severe adverse

reactions. A long search for an appropriate lipid source for clinical nutrition ensued.

Various oil sources including butter oil, coconut oil, cottonseed oil, lard oil, olive oil, sesame seed oil, safflower oil and soybean oil, containing esters of fatty acids (6-22 carbons long) were tried. Also various emulsifying agents including soybean phosphatides, sorbitan monolaurate, polyglycerol esters of fatty acids, gelatin, cholesterol, sodium cholate and egg yolk phosphatides which are necessary to allow solubility of these lipids in an aqueous environment such as the blood stream were employed. (Thompson, S. W. The Pathology of Parenteral Nutrition with Lipids. Springfield, IL: Charles C. Thomas, 1974) This search was at first unsuccessful due to impurities such as high free fatty acids found in these primitive oils and emulsifiers. Over the last thirty years this search has focused on two possible oils and emulsifiers that showed therapeutic potential. The first of these were liquid emulsions composed of cottonseed oil (10 to 20% wt/v), soybean phospholipid (1-5% wt/v) and glycerin (2.25% w/v). Early emulsions of this composition showed a high degree of toxicity in both animals and man. (Meng, H. C. and J. S. Kaley., Effects of Multiple Infusions of a Fat Emulsion on Blood Coagulation, Liver Function, and Urinary Excretion or Steroids in Schizophrenic Patients. J Clin Nutr 16: 156-164, 1965) Since then such emulsions have undergone improvements. Both the oil and emulsifiers have been further characterized and purified and presently appear to provide a therapeutic modality to supply calories to the critically ill. (Lipofundin S. Fat Emulsion for Parenteral Hyperalimentation and Supply of Polyunsaturated Essential Fatty Acids. Germany: B. Braun, 1981) However, due to their notorious past, emulsions of such composition are little used in clinical nutrition.

The second emulsion which evolved during this period was one composed of purified soybean oil (10-20% wt/v), egg yolk phospholipids (1-5% wt/v) and 2.25% w/v glycerin. This emulsion, due to the purified nature of its components, produced clinically acceptable results as a calorie source in clinical nutrition. (Wrentlind, A. Current Status of Intralipid and other Fat Emulsions. pp109-122 in: Fat Emulsions in Parenteral Nutrition. Meng, H. C. and Wilmore, D. W., ed. Chicago, American Medical Association, 1976) This emulsion then established lipid emulsions as a viable nutrition therapy, and several emulsions of this composition are presently on the market. Recent additions to this family of lipid calorie sources are compositions of safflower oil and mixtures of soybean and safflower oils which appear to be viable emulsions as well. (Ament, M. E., R. A. Cannon, and W. J. Byrne. Use of Intravenous Safflower Oil Emulsion (Liposyn 10%) as an Energy Source in Pediatric Patients on TPN. (p165 in Parenteral Nutrition in the Infant Patient. North Chicago, IL: Abbott Laboratories, 1983)) From this historical summary it would appear that the nature of the oil and emulsifier appear to be less important than their purity for their use in clinical nutritions.

As the emulsions were developing, the biochemistry of lipids was also evolving. This resulted in the discovery of the biological essentiality of certain polyunsaturated fatty acids [linoleic acid (C18:2 omega 6), arachidonic acid (C20:4 omega 6)]. (Holman, R. T. How Essential are Essential Fatty Acids. J Amer Oil Chem Soc, 55: 744A-781A, 1978) It was observed that lack of these essential fatty acids produced a clinical syndrome characterized by scaliness and lesions of skin, cessation of growth, renal degeneration, structural and metabolic changes in the central nervous system, increased metabolic rate, weight loss and

finally death. (Caldwell, M. D. Human Essential Fatty Acid Deficiency: A Review in Fat Emulsions in Parenteral Nutrition. Meng, H. C. and Wilmore, D. W., eds. Chicago, IL: Amer Med Assoc, p24, 1978) More recently, the essentiality of **linolenic acid** (C 18:2 omega 3) has been postulated. Deficiencies in this fatty acid cause optical and neurological disturbances. (Neuringer, M., W. E. Connor, C. Van Patten, and L. Bostad. Dietary **Omega 3 Fatty Acid** Deficiency and Visual Loss in Infant Rhesus Monkeys. J Clin Chem 73: 272-276, 1984). These developments further increased the therapeutic utility of lipids in clinical nutrition.

The fat emulsions outlined above have been used successfully both as a calorie and an essential fatty acid source for the last twenty years. (Pelham, D. Rational Use of Fat Emulsions. The Hosp Pharm Forum 10:1, 1981) Problems associated with their use are generally considered to be due to lipid overload. This is when concentrations of lipid in the emulsion or its metabolic products (free fatty acids) are such that the body is unable to metabolize them. (Alexander, C. S. Fat infusions: Toxic Effects and Alterations in Fasting Serum Lipids following Prolonged Use. Arch Intern Med 107: 94-514, 1961) This results in lipid accumulation in various cells, tissued, and organs of the body. (Belin, R. P., B. A. Bivins, J. Z. Jona, V. L. Young. Fat Overload with a 10% Soybean Oil Emulsion. Arch Surg 111: 1391, 1976) High levels in the blood of the emulsion's by-products, free fatty acids, have been shown to cause both cardiac and lung damage. (Soloff, L. A. Arrhythmias Following Infusions of Fatty Acids. Amer Heart J 80: 671, 1970; Broe, P. J., T. J. K. Toung, S. Margolis, S. Permutt and J. L. Cameron. Pulmonary Injury Caused by Free Fatty Acid. Evaluation of steroid and albumin therapy. Surgery 89: 582, 1981)

Fat emulsions are recommended clinically to be used at dosages of 2.5 g/kg/24 hours for adults and up to 4 g/kg/24 hours for children. (TRAVAMULSION 10% I.V. fat emulsion product insert. Deerfield, IL: Travenol Laboratories, 1985) These emulsions contain no more than 5 meq/liter of free fatty acids. The dosage level of these emulsions are recommendations and each patient must be monitored for the build up of emulsions and free fatty acids during infusion to assure safety of such therapies. Extensive studies to assess the metabolism and pharmacokinetics of these emulsions during infusion have been conducted and are well understood at this time. (Cotter, R., L. Martis, F. Cosmas, H. Sargent, C. Taylor, W. Remis, S. Young, W. B. Rowe, and E. Woods. Nonlinear kinetic analysis of the elimination of lipid emulsion administered Intravenously to Dogs. J Paren Ent Nutr (7(3): 244-250, 1983; Cotter, R., L. Martis, F. Cosmas, H. Sargent, C. Taylor, S. Young, W. B. Rowe, and E. Woods. Comparison of the Elimination and Metabolism of 10% TRAVAMULSION and 10% Intralipid Lipid Emulsion in the Dog. J Paren Ent Nutr 8(2): 140-145, 1984; Cotter, R. L. Martis, F. Cosmas, C. Taylor, S. Young, W. B. Rowe, and R. Johnson. Comparison of the Elimination of 10 and 20% TRAVAMULSION Lipid Emulsion from the Blood of Beagle Dogs. Amer J. Clin Nutr 41(5): 994-1001, 1985)

Presently a new generation of lipid emulsions is under development. These emulsions are designed as therapeutic modalities for clinical conditions that have high metabolic energy requirements. These conditions are a result of hormonal and biochemical aberrations that alter normal energy metabolism and shift it into a hypermetabolic state. (Raymond, R., R. Cotter, F. Cosmas, and D. Gibbons. Development of a Chronic Peritoneal Abscess Model in the Dog from Evaluation of Clinical Therapies. Fed Proc 43: 325, 1984) Such states are found in critically

ill patients suffering from trauma, sepsis and burns. (Kinney, J. M. and P. Felig. The Metabolic Response to Injury and Infection. Endocrinology 3: 1963, 1979) These emulsions are composed of medium chain fatty acids (C6 to C12) esterified to glycerol to form medium chain triglycerides which are emulsified with (1-5% wt/v) egg yolk phospholipids to give a final triglyceride concentration of 10 to 20% wt/v. (Cotter, R., F. Cosmas, R. Johnson, B. Rowe, and L. Lin. A Comparison of the Elimination of Four Different Formulations of Parenteral Lipid Emulsions from the Blood Streams of the Beagle Dog. Fed Proc 44: 1146, 1985) These emulsions are of benefit in the hypermetabolic state as they supply twice as much metabolic energy per gram of lipid at a faster rate due to their unique biochemical advantage of carnitine independence, rapid betaoxidation and lack of deposition in organs and adipose tissue as compared to long chain triglycerides (C12-C24). (Cotter, R. C. Johnson, C. A. Taylor, T. Pavline, F. Cosmas, and W. B. Rowe. Metabolic Comparison of a 20% Combination Long and Medium Chain Triglyceride Lipid Emulsion and a 20% Long Chain Emulsion. Fed Proc 43: 848, 1984; Johnson, R. C., S. K. Young, R. Cotter, and W. B. Rowe. Metabolism and Distribution of Medium Chain Triglyceride Lipid Emulsion. Amer J. Clin Nutr 41: 846, 1985) Extensive research has been carried out to develop and characterize these emulsions, illustrating their metabolic advantage. (Young, S. K., R. C. Johnson, R. Cotter, and B. Rowe. Competitive Interaction Between Medium and Long Chain Lipid Emulsions. Fed Proc 43: 865, 1984).

The rapid bioavailability of lipid emulsions creates immediate biological effects and makes them attractive vehicles for acute intravenous therapies. Further studies have also shown that by reducing the phospholipid composition of the emulsion to about 0.4-0.6% a more rapid bioavailability is produced. This rapid bioavailability is produced by creating a more attractive lipid particle for apolipoprotein transfer from high density lipoproteins found in circulating blood. Such apolipoproteins are essential for control of lipid emulsion endothelial receptor binding and activation of hydrolytic enzymes at these receptor sites. The reduction in phospholipids in such emulsions results in a more rapid delivery of the emulsion to metabolism and a release of the biologically active metabolic products. This brings about a rapid biological response to these therapies.

Lipid emulsions containing marine oil have been proposed for the treatment of disorders associated with imbalances of arachidonic acid metabolites. Examples include: autoimmune syndromes; acute and chronic **inflammatory** diseases such as psoriasis and acute respiratory distress syndrome (ARDS); atherosclerosis, stroke, myocardial infarction, deep vein thrombosis and other cardiovascular diseases. The most notable cardiovascular risk factors include surgery, hyperlipidemic states, hypertension (stroke), enhanced platelet responsiveness, vascular lesions and occlusions, vascular spasm and diabetes. Studies have shown that populations (Greenland Eskimos) whose diets are rich in marine products are at considerably reduced risk of developing coronary heart disease. (Editorial. Eskimo diets and diseases. Lancet: 1139-1141, May 21, 1983) Such diets are rich in fatty acids of the omega three (omega 3) family. The three members of this family which appear to play a significant role in this effect are **linolenic acid** (C18:3), eicosapentaenoic acid or EPA (C20:5), and docosahexaenoic acid or DHA (C22:6).(Bang, H. O., J. Dyerberg, and N. Hjorne. The Composition

of Food Consumed by Greenland Eskimos. Acta Med Scand 200: 69-73, 1976)

In the average European and North American diet, linoleic acid (C18:2), an **omega 6 fatty acid**, is the predominantly consumed essential fatty acid, accompanied by low levels of **linolenic acid**. Linoleic acid is converted to arachidonic acid (C20:4), both of which are incorporated into the lipid component of cell membrances and serum, and give rise to metabolites of the omega 6 pathways.

Cold water marine animals contain low concentrations of the essential fatty acid, linolenic, in their tissues and large amount of two other members of the omega 3 family: EPA and DHA. These fatty acids are also incorporated into cell membranes and serum and give rise to metabolites of the omega 3 pathways. The two metabolic pathways containing the **omega 3 fatty acids** are not interchangeable in animals. However, the enzymes which metabolize the omega 6 and omega 3 series seem to be identical.

Most animal cells utilize these fatty acids to form various prostaglandins and leukotrienes. (Spector, A. A., T. L. Kuduce, P. H. Figard, K. C. Norton, J. C. Hoak, and R. L. Czeruionke. Eicosapentaenoic Acid and Prostacyclin Production by Cultured Human Endothelial Cells. J Lipid Res 24: 1595-1604, 1983; Lee, T. H., R. L. Hoover, J. D. Williams, et al. Effect of Dietary Enrichment with Eicosapentaenoic and Docosahexaenoic Acids on in vitro Neutrophil and Monocyte Leukotriene Generation and Neutrophil Function. N Engl J Med 312(19): 1217-1224, May 9, 1985) When fatty acids are released from cell membranes and intracellular pools, the lipoxygenase and cyclooxygenase enzymes mediate the production of various eicosanoids. Although EPA is a relatively poor substrate for cyclooxygenase, it appears to have a high binding affinity and thereby inhibits arachidonic acid conversion by this enzyme. (Needleman, P., A. Raz, M. Minkes, J. A. Ferrendelli, and H. Sprecher. Triene Prostaglandins, Prostacyclin and Thromboxane Biosynthesis and Unique Biological Properties. Proc Nat Acad Sci USA 76: 944, 1979) On the other hand, EPA is a good substrate for the lipoxygenase enzymes. (Terano, T., J. A. Salmon, and S. Moncada. Biosynthesis and biological activity of leukotriene B.sub.5. Prostaglandins 27(2): 217-232, 1984) In either case, EPA would have clinical application in disorders associated with elevated levels of arachidonic acid metabolites (examples: thromboxane B.sub.2 mediated myocardial infarction; (Hay, C. R. M., A. P. Durber, and R. Saynor. Effect of Fish Oil on Platelet Kinetics in Patients with Ischemic Heart Disease. Lancet 1269-1272, June 5, 1982) and leukotrienes in psoriasis. (Brain, S. D., R. D. R. Camp, A. Kobza Black, et al. Leukotrienes C.sub.4 and D.sub.4 in psoriatic skin lesions. Prostaglandins 29(4): 611-619, 1985)

An additional application of the **omega 3 fatty acid** pathway lies in the physiological activities of their cellular products. EPA has been shown to lower platelet activity. (Holme, S., J. H. Brox, H. Krane, and A. Nordoy. The Effect of Albumin Bound Polyunsaturated Fatty Acids on Human Platelets. Throm Haemostas 51(1): 32-36, Stuttgart, 1984) Platelet activation and release is implicated in the pathophysiology of such cardiovascular disorders as atherosclerosis; (Ross, R., and L. Harker, Hyperlipidaemia and atherosclerosis. Science 193: 1094, 1976); thrombosis, (Hornstra, G. Dietary Fats and Arterial Thrombosis: Effects and Mechanism of Action.

Prog Biochem Pharmacol 14: 326-338, 1977); myocardial infarction, (Hay, C. R. M., A. P. Durber, and R. Saynor, Effect of Fish Oil on Platelet Kinetics in Patients with Ischemic Heart Disease, Lancet 1269-1272, June 5, 1982); and shock. (Lefer, A. M. Role of the Prostaglandin-Thromboxane System in Vascular Homeostasis During Shock. Circ Shock G: 297-303, 1979)

Many short-term studies involving the daily administration of some marine products to apparently health human subjects have demonstrated similar findings to those reported for Greenland Eskimos. There is a mild bleeding defect (prolonged bleeding time) and platelet aggregation response to collagen, or ADP is markedly reduced. (Goodnight, S. J., W. C. Harris, and W. E. Connor. The Effects of Dietary **Omega-3 Fatty Acids** on Platelet Composition and Function in Man: A Prospective, Controlled Study. Blood 58(5): 880-885, 1981; Thorngren, M., and A. Gustafson. Effects of 11-week Increase in Dietary Eicosapentaenoic Acid on Bleeding Time, Lipids, and Platelet Aggregation. Lancet: 1190-1193, Nov 28, 1981) In nonhuman primates with advanced atherosclerosis and markedly shortened platelet survival times, the offering of a diet containing EPA resulted in the normalizing of platelet survival times. (Ward, M. V., and T. B. Clarkson. The Effect of a Menhaden Oil Containing Diet on Hemostatic and Lipid Parameters of Nonhuman Primates with Atherosclerosis. Atherosclerosis (in press))

In most normal subjects and patients who consume such diets, total serum cholesterol, very low density lipoprotein cholesterol, and triglycerides are significantly lowered. (Mortensen, J. Z., E. B. Schmidt, A. H. Nielsen, and J. Dyerberg. The Effect of N-6 and N-3 Polyunsaturated Fatty Acids on Hemostasis, Blood Lipids and Blood Pressure. Thromb Haemostas 50(2): 543-546, Stuttgart, 1983; Phillipson, B. E., D. W. Rothrock, W. E. Connor, W. C. Harris, and D. R. Illingworth. Reduction of Plasma Lipids, Lipoproteins, and Apoproteins by Dietary Fish Oils in Patients with Hypertriglyceridemia. N Engl J Med 312(19): 1210-1216, 1985) High density lipoproteins (HDL) cholesterol concentrations may be elevated in some subjects. (Sanders, T. A. B., and M. C. Hochland. A Comparison of the Influence on Plasma Lipids and Platelet Function of Supplements of Omega-3 and Omega-6 Polyunsaturated Fatty Acids. Brit J Nutr 50: 521-529, 1983) This pattern of change would be one thought to be less atherogenic.

Studies with animals have shown that those fed diets containing EPA, as opposed to commercial chows, have significantly lower infarct sizes when their coronary or carotid arteries are ligated. (Culp, B. R., W. E. M. Lands, B. R. Lucchesi, B. Pitt, and J. Romson. The Effect of Dietary Supplementation of Fish Oil on Experimental Myocardial Infarction. Prostaglandins 20(6): 1021-1031, 1980; Black, K. L., B. Culp, D. Madison, O. S. Randall, and W. E. M. Lands. The Protective effects of dietary fish oil on focal cerebral infarction. Prostaglandins & Med 3: 257-268, 1979). The difference is thought to be due to a reduced oxygen demand on the part of the affected tissue. This would support the findings from studies with nonhuman primates whereby a diet containing EPA had a sparing effect upon the onset and extent of myocardial ischemia after isoproterenol stress tests. (Ward, M. W. Unpublished finding, Bowman Gray School of Medicine, Winston-Salem, NC) In studies with human subjects fed marine products, both blood pressure and blood pressure response to norepinephrine fell significantly. (Lorenz, R., U. Spengler, S. Fischer, J. Duhm, and P. C. Weber. Platelet Function, Thromboxane Formation and Blood Pressure Control During Supplementation of the Western Diet with Cod Liver Oil. Circulation 67(3): 504-511,

1983).

Change in fatty acid composition of blood cell membranes and serum may explain some of the aforementioned physiological observations. With the ingestion of a marine diet, the **omega 3 fatty acids** increase markedly at the expense of the **omega 6 fatty acids.**

There may even be other benefits to fish products. Certain mice that die at an early age of autoimmune disease have been given prostaglandin E.sub.1 (PGE.sub.1) or menhaden oil diets and exhibited markedly longer lifespans and a virtual disappearance of immune mediated glomerulonephritis. (Kelley, V. E., A. Winkelstein, S. Isui, and F. J. Dixon. Prostaglandin E.sub.1 Inhibits T-Cell Proliferation and Renal Disease in MRL/1 Mice. Clin Immunology & Immunopathology 21: 190-203, 1981; Prickett, J. D., D. R. Robinson, and A. D. Steinberg. Dietary Enrichment with the Polyunsaturated Fatty Acid Eicosapentaenoic Acid Prevents Proteinuria and Prolongs Survival in NZB X NZW F.sub.1 Mice. J Clin Invest 68: 556-559, 1981) Fish oil was also found to be beneficial in a marine model of anyloidosis. (Hayes, K. D., E. Cathcart, C. A. Leslie, and S. N. Meydani. Dietary Fish Oil Alters Prostaglandin Metabolism to Decrease Platelet Aggregation in Monkeys and Anyloidosis in Mice. Proc of Conf on **Omega-3 Fatty Acids**. Reading, England: Reading University, 131-132, Jul 16-18, 1984).

The beneficial effects of fish oils in **inflammatory** disorders stem, at least in part, from the interaction of EPA and arachidonic acid with the enzyme lipoxygenase in **inflammatory** cells (neutrophils and monocytes). In the presence of EPA these cells produce less Leukotriene B.sub.4 (a major component of **inflammatory** response) and small amounts of Leukotriene B.sub.5. (Lee, T. H., R. L. Hoover, J. D. Williams, et al. Effect of Dietary Enrichment with Eicosapentaenoic and Docosahexaenoic Acids on in vitro Neutrophil and Monocyte Leukotriene Generation and Neutrophil Function. N Engl J. Med 312(19): 1217-1224, 1985) LTB.sub.5 is at least 30 times less potent than LTB.sub.4 in causing aggregation, chemokinesis and degranulation of human neutrophils in vitro. The potency of LTB.sub.5 in potentiating bradykinin-induced plasma exudation, which is probably attributable to its leukotactic activity, is as least 10 times lower than that of LTB.sub.4. (Terano, T., J. A. Salmon, and S. Moncada. Biosynthesis and Biological Activity of Leukotriene B.sub.5. Prostaglandins 27(2): 217-232, 1984)

U.K. patent application GB No. 2 139 889A discloses an emulsion for intravenous use which contains a fatty acid containing 20-22 carbon atoms or an ester of the fatty acid, a vegetable oil, an emulsifier and water.

It is an object of this invention to provide a lipid emulsion for intravenous therapy and treatment of thrombotic disease. It is a further object of this invention to provide an emulsion which inhibits formation of certain prostaglandins. It is a further object of this invention to provide such an emulsion wherein the concentrations of free fatty acids are below toxic levels.

Other objects appear hereinafter.

SUMMARY

We have found that lipid emulsions of marine oils comprising high

concentrations of **omega-3-fatty acid** esters and low concentrations of free fatty acids are therapeutic when administered intravenously for the treatment of thrombotic disease states.

Specifically, a lipid emulsion for parenteral use is provided comprising an emulsifier, water, and a marine oil comprising an **omega-3 fatty acid** ester, in which the concentration of free fatty acid in the emulsion is below about 5 meq/l. Preferably, the concentration of marine oil in the emulsion is between 5 and 50% (wt/v).

Specifically, marine oil containing **omega-3 fatty acid** esters is predominantly made of acids of 12-26 carbon atoms each, for example, esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), typically as a mixture, although pure species may be used as well. Preferably, the ester of EPA may be present in the marine oil in a concentration of 10 to 100% by weight.

Typical esters of EPA, DHA, or other unsaturated acids of 12-26 carbons are the glyceryl esters of naturally occurring fats.

The emulsifier may be any physiologically appropriate emulsifier, being typically selected from the group consisting of egg yolk phosphatide, soy phosphatide, purified egg yolk lecithin, purified soy lecithin, and other purified phospholipids. The emulsifier concentration may typically range from 0.2 to 1.5%, and preferably about 0.3 to 0.8% for optimum production of rapid bioavailability of EPA and DHA.

The term "**omega-3 fatty acid** ester" is defined to mean that the particular fatty acid included in the ester has a double bond occuring at the third position from the methyl end of the fatty acid. Likewise, the term "omega-6" implies that the first double bond in the molecule of the fatty acid in question occurs at the sixth position from the methyl end.

Preferably the lipid emulsions of this invention are free of vegetable oils and acids derived therefrom.

DETAILED DESCRIPTION OF THE INVENTION

All percentages in this application refer to weight/volume unless otherwise noted.

The intravenous lipid emulsions of this invention comprise marine oil, an emulsifier, and water.

The marine oils to be used herein are those which are preferably highly purified. These oils have a high concentration of fatty acid esters relative to free fatty acids. Examples of such oils include:

menhaden oil,

salmon oil,

sardine oil,

and other fish oils from cold water ocean fish.

The amount of oil to be used in the emulsion will depend upon the dosage, the percentage of fatty acid esters in the oil, and the total

lipid concentration of the emulsion. Therapeutic dosages will be dependent upon body weight and infusion duration. The **omega 3 fatty acid** ester content of the oil will also vary depending upon the oil source. Concentrations will range from 10 to 100% and preferably at least 30%. Free fatty acid concentration of total lipid emulsion should be below 5 meq/l. Concentration of the marine oil in the emulsion will vary between 5 to 50%. Preferred concentrations are between 10 to 20%; concentrations of emulsifiers will vary accordingly.

Emulsifiers which are useful in this invention include egg yolk phosphatide, soybean phosphatide, egg yolk lecithin, soybean lecithin and other purified phospholipids. Concentrations of the emulsifiers are dependent upon the amount of oil in the emulsion. Concentrations may range from 0.1 to 6%. For each additional 10% increase in oil, emulsifier concentration will increase approximately 0.4 to 1.2%. Preferred concentrations are about 0.4 to 1.2% where volume of oil is between 10 to 20%.

Various osmotic agents may also be added to the emulsion. Examples of such osmotic agents include glycerin, glucose, sucrose, sorbitol, protein and sodium acid phosphates. The osmolarity of this solution preferrably ranges between 280 to 300 milliosmoles. The remainder of the emulsion comprises mostly water and other optional additives.

The lipid particles in the emulsion will have a diameter of less than about 0.75 .mu.m and preferably less than about 0.5 .mu.m. The emulsions will be sterile and ordinarily are packaged in glass or plastic containers. They can be made by known methods. For example, see U.S. Pat. No. 3,169,094 and European Patent Application No. 0071995. The emulsions herein are packaged and stored in hermetically sealed containers for long and short-term storage.

DETD EXAMPLE 1

In a suitable vessel, 1.0 to 2.0 Kg of marine oil containing 15-30% glycerol ester of eicosapentaenoic acid (EPA) and 15-25% glycerol ester of docosahexaenoic acid (DHA), 120 g of purified egg phospholipids, 225 g of glycerol, USP, (as an osmotic agent) and water for injection USP are mixed to produce an emulsion having a 2.25% glycerol concentration and a 10 to 20% marine oil concentration. This emulsion is then homogenized repeatedly at high pressure to produce an emulsion of mean particle diameter of less than 0.75 .mu.m. During the process, the pH of the emulsion is adjusted to a physiological range with sodium hydroxide.

The final volume is adjusted, if necessary with water for injection, USP, to 10 liters, and the emulsion is filtered into glass containers and heat sterilized by the normal procedure.

EXAMPLE II

A 10% lipid emulsion of the type of Example 1 was administered, via a cephalic vein intravenously, to each of 6 dogs, continuously over an 8 hour period, at a rate of 40 mg EPA/kg/hr (2.5 ml/kg/hr). Each of the same 6 dogs received similar 8 hour infusions of Liposyn 10% Safflower oil lipid emulsion (Abbott Laboratories, North Chicago) and physiological saline (Travenol) in equivalent volumes to those administered for the Example 1 lipid emulsion (2.5 ml/kg/hr). There was a 21 day washout period between each infusion to the same dog. The order

of treatments was randomized.

The Example 1 lipid emulsion contained 10 gm marine oil per 100 ml emulsion, and 16.42 mg EPA per ml of emulsion. From the time of production until the time of infusion, the Example 1 lipid emulsion was stored at approximately 4.degree. C. During the infusion, the emulsion stood at room temperature.

Citrated whole blood samples were drawn from each dog at the following times: pre-infusion, and 2, 4, 6, 8, 10, 24, and 48 hours following the start of infusion. Assays completed with these blood samples included whole blood platelet aggregation to adenine-5-diphosphate (ADP) and collagen, prothrombin time, and activated partial thromboplastin time. Whole blood platelet counts were also measured at the above listed time periods, using whole blood collected into EDTA.

After the administration of the Example 1 lipid emulsion, dog platelets challenged with 8 .mu.M adenine-5-diphosphate (ADP) were inhibited 80%, 29.8% and 21% at 8, 24, and 48 hours after beginning infusion, respectively, when compared to pre-infusion responses. When these same platelets were challenged with 2 .mu.g/ml of acid soluble collagen, they were inhibited 72.9%, 25.8% and 20% at 8, 24, and 48 hours after beginning infusion, respectively, when compared to pre-infusion responses. After the administration of Liposyn, dog platelet responses to both ADP and collagen were at or above (hyperactive) pre-infusion values at both 24 and 48 hours after beginning infusion. Platelet counts were unaltered by the infusion of the Example 1 lipid emulsion, Liposyn, or saline.

A cuticle bleeding time test was used in this dog study. This is an "open bleed" assessment of hemostatic capacity in which a toenail is severed in a manner sufficient to transect the vascular supply to that nail. The test measures the length of time required to cease bleeding. These tests were completed on each dog pre-infusion, and at 8 and 24 hours after beginning infusion. Cuticle bleeding times of dogs receiving the Example 1 lipid emulsion were increased 158% and 152% above pre-infusion values at the 8 and 24 hour time periods, respectively. These increases were consistent with the inhibition of platelet function. Dogs receiving Liposyn had bleeding times decrease 14% and 22% below pre-infusion values at the 8 and 24 hours time periods, respectively. These decreases were consistent with the platelet aggregation responses at the same time periods.

Blood coagulation tests revealed significant prolongations of both prothrombin times and activated partial thromboplastin times with blood samples collected from dogs receiving the Example 1 lipid emulsion. These changes were not seen with the infusion of saline or Liposyn.

EXAMPLE III

A 10% lipid emulsion made as in Example 1 was administered, via a saphenous vein intravenously, to each of 6 African Green Monkeys, continuously over a six hour period, at a rate of 125 mg EPA/kg/hr (5 ml/kg/hr). Each of the same six monkeys received similar six hour infusions of 10% lipid emulsion containing soybean oil (TRAVAMULSION.RTM., Travenol Laboratories, Inc.) in equivalent volumes to those administered for the EPA lipid emulsion (5 ml/kg/hr). There was a twenty-one day washout period between each infusion in the same monkey.

The Example 1 lipid emulsion contained 10 gm of marine oil per 100 ml emulsion, and 25 mg EPA/ml of emulsion. From the time of production until the time of infusion, the Example 1 lipid emulsion was stored at approximately 4.degree. C. During the infusion, the emulsion stood at room temperature.

Citrated whole blood samples were drawn from each monkey pre-infusion, and at 6, 12, and 24 hours after beginning infusion. These samples were used to measure whole blood platelet aggregation to acid soluble collagen, and thromboxane B.sub.2 release by platelets after platelet aggregation to collagen. Whole blood platelet counts were also measured at the above-listed time periods, using whole blood collected into EDTA.

Platelet counts remained unchanged for both treatments. The Example 1 lipid emulsion and TRAVAMULSION.RTM. lipid emulsion were comparable in effect 6 hours after beginning infusion, when comparing platelet aggregation responses and thromboxane B.sub.2 release values. EPA lipid emulsion was significantly more effective than TRAVAMULSION.RTM. lipid emulsion in reducing platelet function at both 12 and 24 hours after beginning infusion. The following is a summary of those responses:

Percent of Pre-infusion African Green Monkey Platelet Function After Intravenous Lipid Emulsion

	EPA lipid emulsion		TRAVAMULSION	
Hours after beginning infusion	platelet aggregation	thromboxane release	platelet aggregation	thromboxane release
1 .mu.g/ml Collagen				
6	22.5%	45.7%	60.9%	50.7%
12	14.5	22.8	77.4	57.3
24	25.1	40.2	109.6	99.9
2 .mu.g/ml Collagen				
6	53.4	51.7	86.9	40.3
12	30.5	30.6	108.3	59.0
24	45.0	46.1	123.2	98.0

CLM What is claimed is:

1. A lipid emulsion for parenteral use comprising an emulsifier, water and a marine oil comprising at least one **omega 3 fatty acid** ester wherein the concentration of free fatty acid in the emulsion is below about 5 meq/l and wherein the concentration of marine oil is between about 5% to about 50%.

2. The emulsion of claim 1 wherein the marine oil contains at least 30% by weight of a combination of esters of eicosapentaenoic acid and docosahexaenoic acid.

3. The emulsion of claim 1 wherein the concentration of the ester of eicosapentaenoic acid in the marine oil is between about 10% to about 100%.

4. The emulsion of claim 1 wherein the emulsifier is selected from the group consisting of egg yolk phosphatide, soy phosphatide, purified egg yolk lecithin, purified soy lecithin and other purified phospholipids.

5. The emulsion of claim 1 wherein the emulsifier concentration is either 1.2%, 0.6% or 0.4%, the latter two being the most effective in producing rapid bioavailability of eicosapentaenoic acid and docosahexaenoic acid.

6. The emulsion of claim 6 wherein the osmotic agent is selected from the group containing glycerin, glucose and sucrose, sorbitol, physiologically aceptable sodium phosphate.

7. The emulsion of claim 1 further comprising an osmotic agent.

8. The emulsion of claim 1 in which essentially all lipid particles present have a diameter of less than 0.5 microns.

9. The emulsion of claim 1 having an osmolarity of 280 to 300 milliosmoles.

10. The emulsion of claim 1 wherein the marine oil is selected from the group comprising menhaden oil, salmon oil and sardine oil.

11. A lipid emulsion for intravenous administration use and effective for inhibiting platelet function comprising from 0.2 to 1.5% of an emulsifier selected from the group consisting of egg yolk phosphatide, soy phosphatide, purified egg yolk lecithin, and purified soil lecithin,
from 5 to 50% of a marine oil comprising at least 30% of **omega -3 fatty acid** esters of glycerol, and
water, essentially all lipid particles in the emulsion having a diameter
of less than 0.75 microns.

12. The lipid emulsion of claim 11 in which the marine oil contains at least 30% by weight of a combination of glycerol esters of eicosapentaenoic acid and docosahexaenoic acid.

13. The lipid emulsion of claim 12 in which the concentration of marine oil present is from 10 to 20%.

14. The lipid emulsion of claim 13 in which an osmotic agent is present selected from the group consisting of glycerin, glucose, sucrose, sorbitol, physiologically acceptable proteins, and sodium acid phosphate.

15. The lipid emulsion of claim 14 in which sufficient osmotic agent is present to provide an osmolarity of 280 to 300 milliosmoles.

16. The lipid emulsion of claim 15 in which less than 5 meq/l of free fatty acids are present.

17. A method of rapidly inhibiting platelet function in an animal said method comprising intravenously administering to said animal a platelet inhibiting effective amount of a lipid emulsion comprising an emulsifier, water and a marine oil comprising at least one omega-3 fatty
ester wherein the concentration of free fatty acid in the emulsion is below about 5 meq/l.

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ARTU 123
CAS INDEXING IS AVAILABLE FOR THIS PATENT.